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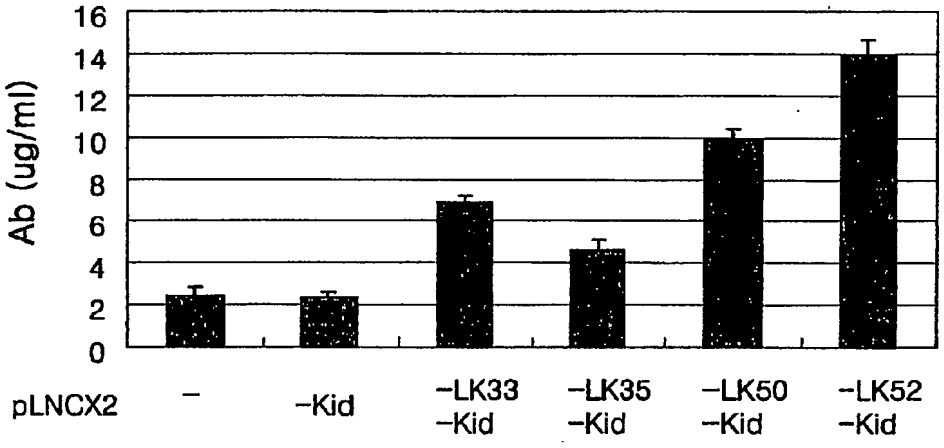

(57) Abstract: Disclosed are a polypeptide capable of increasing the monoclonal antibody production in a eukaryotic cell, a nucleic acid encoding same and a eukaryotic cell transformed with said nucleic acid. The polypeptide and the nucleic acid can be used to increase the production of a monoclonal antibody useful as a medicament for treating various diseases, in a eukaryotic cell.

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POLYPEPTIDE INCREASING THE MONOCLONAL ANTIBODY PRODUCTION, NUCLEIC ACID ENCODING SAME AND USE THEREOF

Field of the Invention

The present invention relates to a polypeptide increasing the monoclonal antibody production in a eukaryotic cell, a nucleic acid encoding same and a eukaryotic cell transformed with the polypeptide or nucleic acid.

Background of the Invention

Since the first production of the human growth hormone by DNA recombination technology in early 1980's, numerous recombinant proteins have been developed and used for treating various diseases. Further, herceptin, a drug for breast cancer approved by FDA (USA) in 1998, has not only established that monoclonal antibody can be effective as a drug, but it also expanded the conventional concept for drug. Thereafter, drugs using monoclonal antibody have been developed for treating cancers and other diseases. Such protein drugs, specifically, monoclonal antibody drugs have diversified with the progress in the field of biology.

Except for a few, e.g. growth hormone, these drugs have to be produced in an animal cell to keep treatment titer at certain level because the protein must go through post-translational modification such as proteolytic cleavage, phosphorylation and glycosylation on specific amino acid to keep in vivo activity which is impossible or inefficient in bacteria or yeast.

With the increase in the number of clinical tests of monoclonal antibodies allowed as useful drugs, demand for mass culture facilities for the protein drugs production has risen explosively (*see* Dove A, *Nature Biotech.* (2001) 19:117-120). The solution for the problem is the expansion of culture facilities and increase of yield rate using present facilities (*see* Fussenegger M, *et al., Trends Biotechnol.* (1999) 17:35-42).

There have been two approaches taken to increase yield rate, one study being related increasing unit production in one cell (Lim L.H. *et al.*, *Biochem. Biophys. Res. Commun.* (2004) 316:991-996) while the other study being related to optimizing the condition of a growth medium to make more cells grow in identical volume (Lee G. M. *et al.*, *J. Biotechnol.* (1999) 69:85-93). The followings exemplify the former approach: a reconstructing vector for the production of target proteins so that it has optimal and maximal productivity (Ted, H. J. *et al.*, *Nature Biotech.* (2003) 21:553-558); developing methods for the fast screening of a clone having high productivity after gene amplification (Charles, G. *et al.*, *Biotechnol.&Bioeng.* (2002) 80:670-676); and controlling proliferation of a clone having high productivity or preventing its apoptosis (Lai, D. *et al.*, *Biotechnol.&Bioeng.* (2004) 85:20-28). These attempts aim at increasing gene expression of target protein.

Cells being used for producing recombinant protein are a CHO cell from Chinese Hamster Ovary, a NS-O as a hybridoma cell of a mouse and a Per.C6 as a human cell, and a CHO cell is the most popular for the production of proteins for medical treatment. The production of a recombinant protein in a CHO cell is maximized by gene amplification using dihydrofolate reductase (DHFR) and methotrexate (MTX) (Kim, S. J., *et al.*, *Biotechnol.&Bioeng.* (1998) 58:73-84).

Aforementioned attempts are about increasing the number of mRNA of genes by amplifying the number of genes in a cell. However, protein production will not be enhanced as much unless the capacity of the intracellular systems working at post-transcriptional process such as translation, post-translational modification and secretion are increased as much as mRNA. Actually, the increase in protein production reaches its plateau once the number of genes is increased more than some point (Schröder, M. *et al.*, *Biotechnol.&Bioeng.* (1997) 53:547-559). Therefore, if this bottleneck problem is resolved by increasing the number of proteins involved in the critical step of post-transcriptional process, notable increase in the protein production can be achieved.

The present invention is to correlate transcriptional regulatory technique of a gene with increase of the intracellular protein production using an artificial transcription factor made of zinc finger. There are many reports showing that

increase or decrease in the specific protein expression is caused by the use of a zinc finger (Zhang L, *et al.*, *J Biol Chem.* (2000) 275:33850-33860; Liu PQ, *et al.*, *J Biol Chem.* (2001) 276:11323-11334; Beerli RR, *et al.*, *Proc Natl Acad Sci USA.* (2000) 97:1495-1500; Beerli RR, *et al.*, *J Biol Chem.* (2000) 275:32617-32627; and Bae K.-H. *et al.*, *Nature Biotech.* (2003) 21:275-280). However, these examples were intended to regulate the transcription of a gene using a zinc finger binding to a promoter of specific gene.

The present inventors have endeavored to develop, a method for increasing the production of a specific protein by regulating an unspecified gene using a library of artificial transcription factors having a random array of zinc finger domains (ZFDs).

Summary of the Invention

Accordingly, it is an object of the present invention to provide a polypeptide increasing the monoclonal antibody production in a eukaryotic cell and a nucleic acid encoding same.

It is another object of the present invention to provide a eukaryotic cell transformed with the polypeptide or nucleic acid.

It is further object of the present invention to provide a method for increasing the monoclonal antibody production in the eukaryotic cell.

In accordance with one aspect of the present invention, there is provided a polypeptide capable of increasing the amount of a monoclonal antibody produced by a eukaryotic cell relative to a cell that does not include the polypeptide, wherein the polypeptide comprises a DNA binding domain that includes a plurality of zinc finger domains and a nucleic acid encoding same.

In accordance with another aspect of the present invention, there is provided a eukaryotic cell containing a gene encoding a monoclonal antibody and the nucleic acid comprising a sequence encoding the polypeptide.

In accordance with further aspect of the present invention, there is provided a method for increasing the monoclonal antibody production in a eukaryotic cell comprising the step of introducing the polypeptide or the nucleic

acid into the cell.

Brief Description of the Drawings

The above and other objects and features of the present invention will become apparent from the following description of the invention taken in conjunction with the following accompanying drawings, which respectively show:

Fig. 1: a schematic diagram of the procedure of preparing pLNCX1, pLNCX2 and pLNCX2-$(ZFD)_4$-Kid library;

Fig. 2: a schematic diagram of screening a zinc finger protein capable of increasing monoclonal antibody production in a mammal, including the procedures of virus production and enzyme-linked immunosorbent assay (ELISA);

Fig. 3: a graph showing increase of the monoclonal antibody production in AKA cell by LK33-Kid, LK35-Kid, LK50-Kid and LK52-Kid, as compared with that in the control cells (LNCX2-Kid and LNCX2);

Fig. 4: a graph showing increase of the monoclonal antibody production in AKA cell by LK52-Kid and its mutant (mLK52-Kid) according to the culture period, as compared with that in the control cell (LNCX2-Kid);

Fig. 5: a sequence of a plasmid p3 including one ZFD;

Fig. 6: a sequence of a pLNCX2-Kid;

Fig. 7: a sequence of pLNCX2-$(ZFD)_4$-Kid, wherein (*ApaI*/*ClaI*) represents a deleted restriction enzyme region, and F1 to F4 represent a zinc finger domain, respectively.

Fig. 8: a schematic diagram of the procedure of mutant insertion to LK52 in pLNCX2-LK52-Kid, wherein "* vertical line" represents a position of a mutation; and

Fig. 9: a graph of quantitative analysis by RT-PCR of mRNA of a monoclonal antibody in AKA cell having Zinc Finger Proteins (ZFPs).

Detailed Description of the Invention

A polypeptide of the present invention, which is capable of increasing the amount of a monoclonal antibody produced by a eukaryotic cell, preferably, a mammalian cell, relative to a cell that does not include the polypeptide, comprises a DNA binding domain that includes a plurality of zinc finger domains (ZFDs).

ZFDs are small polypeptide domains of approximately 30 amino acid residues in which there are four amino acids, either cysteine or histidine, appropriately spaced such that they can coordinate a zinc ion (For reviews, see, e.g., Klug and Rhodes, (1987) *Trends Biochem. Sci.*12:464-469(1987); Evans and Hollenberg, (1988) *Cell* 52:1-3; Payre and Vincent, (1988) *FEBS Lett.* 234:245-250; Miller *et al.,* (1985) *EMBO J.* 4:1609-1614; Berg, (1988) *Proc. Natl. Acad. Sci. U.S.A.* 85:99-102; Rosenfeld and Margalit, (1993) *J. Biomol. Struct. Dyn.* 11:557-570). Hence, zinc finger domains can be categorized according to the identity of the residues that coordinate the zinc ion, e.g., as the Cys_2-His_2 class, the Cys_2-Cys_2 class, the Cys_2-CysHis class, and so forth. The zinc coordinating residues of Cys_2-His_2 zinc fingers are typically spaced as follows: NH_2-X_a-X-C-X_{2-5}-C-X_3-X_a-X_5-ψ-X_2-H-X_{3-5}-H-COOH, where ψ (psi) is a hydrophobic residue (Wolfe *et al.*, (1999) *Annu. Rev. Biophys. Biomol. Struct.* 3:183-212), wherein "X" represents any amino acid, wherein X_a is phenylalanine or tyrosine, the subscript indicates the number of amino acids, and a subscript with two hyphenated numbers indicates a typical range of intervening amino acids. Typically, the intervening amino acids fold to form an anti-parallel β-sheet that packs against an α-helix, although the anti-parallel β-sheets can be short, non-ideal, or non-existent. The fold positions the zinc-coordinating side chains so they are in a tetrahedral conformation appropriate for coordinating the zinc ion. The base contacting residues are at the N-terminus of the finger and in the preceding loop region.

For convenience, the primary DNA contacting residues of a zinc finger domain are numbered: -1, 2, 3, and 6 based on the following example:

```
                    -1 1 2 3 4 5 6
Xa-X-C-X2-5-C-X3-Xa-X-C-X-S-N-Xb-X-R-H-X3-5-H (SEQ ID
```

NO: 1),

where X_a is phenylalanine or tyrosine, and X_b is a hydrophobic residue. As noted in the example above, the DNA contacting residues are Cys (C), Ser (S), Asn (N), and Arg (R). The above motif can be abbreviated CSNR. As used herein, such abbreviation refers to a particular polypeptide sequence. Where two sequences have the same motif, a number may be used to indicate the sequence. In certain contexts where made explicitly apparent, the four letter abbreviation refers to the motif in general.

The term "zinc finger protein" refers to any protein that includes a zinc finger domain. A zinc finger protein typically includes a tandem array of at least two zinc finger domains.

The zinc finger domain (or "ZFD") is one of the most common eukaryotic DNA-binding motifs, found in species from yeast to higher plants and to humans. By one estimate, there are at least several thousand zinc finger domains in the human genome alone, possibly at least 4,500. Zinc finger domains can be isolated from zinc finger proteins. Non-limiting examples of zinc finger proteins include CF2-II, Kruppel, WT1, basonuclin, BCL-6/LAZ-3, erythroid Kruppel-like transcription factor, Sp1, Sp2, Sp3, Sp4, transcriptional repressor YY1, EGR1/Krox24, EGR2/Krox20, EGR3/Pilot, EGR4/AT133, Evi-1, GLI1, GLI2, GLI3, HIV-EP1/ZNF40, HIV-EP2, KR1, ZfX, ZfY, and ZNF7.

Computational methods described below can be used to identify all zinc finger domains encoded in a sequenced genome or in a nucleic acid database. Any such zinc finger domain can be utilized. In addition, artificial zinc finger domains have been designed, e.g., using computational methods (e.g., Dahiyat and Mayo, (1997) *Science* 278:82-7).

It is also noteworthy that at least some zinc finger domains bind to ligands other than DNA, e.g., RNA or protein. Thus, a chimera of zinc finger domains or of a zinc finger domain and another type of domain can be used to recognize a variety of targets compounds, not just DNA.

U.S. Patent Application Serial No. 60/374,355, titled "Zinc Finger Domain Libraries," and filed April 22, 2002 describes exemplary zinc finger domains, which can be used to construct an artificial zinc finger protein. See also the Table 1.

Preferably, the DNA binding domain comprises at least 2 ZFDs selected from the group consisting of the following amino acid sequences:

$CX_{(2-5)}CXXXBXRXXHJXTHX_{(3-5)}H$ (SEQ ID NO: 2);
$CX_{(2-5)}CXXXBXQXXHJXRHX_{(3-5)}H$ (SEQ ID NO: 3);
$CX_{(2-5)}CXXXBXQXXNJXKHX_{(3-5)}H$ (SEQ ID NO: 4);
$CX_{(2-5)}CXXXBXQXXSJXRHX_{(3-5)}H$ (SEQ ID NO: 5);
$CX_{(2-5)}CXXXBXQXXNJXRHX_{(3-5)}H$ (SEQ ID NO: 6);
$CX_{(2-5)}CXXXBXQXXNJXIHX_{(3-5)}H$ (SEQ ID NO: 7);
$CX_{(2-5)}CXXXBXRXXKJXRHX_{(3-5)}H$ (SEQ ID NO: 8);
$CX_{(2-5)}CXXXBXQXXHJXVHX_{(3-5)}H$ (SEQ ID NO: 9); and
$CX_{(2-5)}CXXXBXQXXHJXQHX_{(3-5)}H$ (SEQ ID NO: 10),

where B is phenylalanine or tyrosine; J is a hydrophobic amino acid; and X is any amino acid.

More preferably, the polypeptide of the present invention further comprises a domain selected from the group consisting of a transcription activation domain, a transcription repression domain, a protein transduction domain (PTD) and a combination thereof bound to the ZFD-including DNA binding domain.

In the present invention, each zinc finger domain is named after its DNA contacting amino acid residues, i.e., amino acid residues at positions –1, 2, 3 and 6 along the alpha helix structure. For example, when the amino acid residues at positions –1, 2, 3 and 6 along the alpha helix structure are arginine (R), aspartic acid (D), histidine (H) and threonine (T), the zinc finger domain is represented as RDHT.

The term "DNA contacting residue" as used herein refers to the four amino acid positions of zinc finger domains that structurally correspond to the positions of amino acids arginine 73, aspartic acid 75, glutamic acid 76, and arginine 79 of finger 3 of zinc finger protein zif268 (Kim and Pabo, *J. Biol. Chem.*, (1997) 272(47): 29795-800). Said positions are also referred as positions –1, 2, 3 and 6, respectively. Among these four DNA contacting residues, the amino acid residues at positions –1, 3 and 6 play a key role for DNA recognition and the amino acid residue at position 2 plays an auxiliary role.

The polypeptide of the present invention may comprises at least 2, preferably 2 to 6 zinc finger domains selected from the group consisting of the zinc finger domains having the amino acid sequence of SEQ ID Nos. 2 to 10, and each zinc finger domain can be a wild type, non-wild type or a combination

thereof. Additionally, the zinc finger domains may include various conventional linkers e.g. a peptide linker between the domains.

The utility and design of linkers are well known in the art. A particularly useful linker is a peptide linker that is encoded by a nucleic acid. Thus, one can construct a synthetic gene that encodes a first DNA binding domain, the peptide linker, and a second DNA binding domain. This design can be repeated in order to construct large, synthetic, multi-domain DNA binding proteins. PCT WO 99/45132 and Kim and Pabo ((1998) *Proc. Natl. Acad. Sci. USA* 95:2812-7) describe the design of peptide linkers suitable for joining zinc finger domains.

Additional peptide linkers are available that form random coil, α-helical or β-pleated tertiary structures. Polypeptides that form suitable flexible linkers are well known in the art (see, e.g., Robinson and Sauer (1998) *Proc Natl Acad Sci U S A.* 95:5929-34). Flexible linkers typically include glycine, because this amino acid, which lacks a side chain, is unique in its rotational freedom. Serine or threonine can be interspersed in the linker to increase hydrophilicity. In additional, amino acids capable of interacting with the phosphate backbone of DNA can be utilized in order to increase binding affinity. Judicious use of such amino acids allows for balancing increases in affinity with loss of sequence specificity. If a rigid extension is desirable as a linker, α-helical linkers, such as the helical linker described in Pantoliano *et al.* (1991) *Biochem.* 30:10117-10125, can be used. Linkers can also be designed by computer modeling (see, e.g., U.S. Patent. No. 4,946,778). Software for molecular modeling is commercially available (e.g., from Molecular Simulations, Inc., San Diego, CA). The linker is optionally optimized, e.g., to reduce antigenicity and/or to increase stability, using standard mutagenesis techniques and appropriate biophysical tests as practiced in the art of protein engineering, and functional assays as described herein.

For implementations utilizing zinc finger domains, the peptide that occurs naturally between zinc fingers can be used as a linker to join fingers together. A typical such naturally occurring linker is: Thr-Gly-(Glu/Gln)-(Lys/Arg)-Pro-(Tyr/Phe) (SEQ ID NO: 11) (Agata *et al.*, (1998) *Gene* 213:55-64).

More preferably, the DNA binding domain of the inventive polypeptide includes, in the order of N-terminal to C-terminal, the first, second, third and

fourth zinc finger domains, wherein

(1) the DNA contacting residues at positions –1, 3 and 6 of the first zinc finger domain are R, H and T, respectively; those of the second zinc finger domain are Q, H and R, respectively; those of the third zinc finger domain are Q, N and K, respectively; and those of the fourth zinc finger domain are Q, S and R, respectively;

(2) the DNA contacting residues at positions –1, 3 and 6 of the first zinc finger domain are R, H and T, respectively; those of the second zinc finger domain are Q, N and R, respectively; those of the third zinc finger domain are Q, H and R, respectively; and those of the fourth zinc finger domain are Q, N and I, respectively;

(3) the DNA contacting residues at positions –1, 3 and 6 of the first zinc finger domain are Q, H and R, respectively; those of the second zinc finger domain are R, K and R, respectively; those of the third zinc finger domain are R, H and T, respectively; and those of the fourth zinc finger domain are Q, H and R, respectively; or

(4) the DNA contacting residues at positions –1, 3 and 6 of the first zinc finger domain are R, H and T, respectively; those of the second zinc finger domain are Q, H and V, respectively; those of the third zinc finger domain are Q, H and Q, respectively; and those of the fourth zinc finger domain are Q, S and R, respectively.

The most preferable polypeptides of the present invention have the amino acid sequence of any one of SEQ ID NOs: 12 to 15, and are designated as LK33, LK35, LK50 and LK52, respectively.

The polypeptide of the present invention may further comprise a domain selected form the group consisting of a transcription activation domain, a transcription repression domain, a protein transduction domain (PTD) and a combination thereof to induce the increase in the monoclonal antibody production in a eukaryotic cell.

Exemplary transcription activation domains include a Gal4 activation domain of yeast (Laughon, A. and Gesteland, R. F. *Mol. Cell. Biol.*, (1984) 4: 260-267; Amino acid No. 768-878 of NCBI accession number AAA45766), a VP16 domain of herpes simplex virus (Pellett, P. E. *et al.*, *Proc. Natl. Acad. Sci. USA*

(1985) 82:5870-5874; Amino acid No. 402-479 of NCBI accession number AAA45766) and a p65 of mammalian cell (Nolan G. P. *et al.*, *Cell* (1991) 64:961-969; Amino acid No. 275-535 of NCBI accession number NP_068810), but not limited thereto.

Examples of the eukaryotic transcription repression domains include repression domains from Kid, UME6, ORANGE, groucho, and WRPW (Dawson et al., Mol. Cell Biol. (1995) 15: 6923-6931). Specifically, it can be a "KRAB" domain of Kid protein of rat (Witzgall R. *et al.*, *Proc. Natl. Acad. Sci. USA* (1994) 91: 4514-4518) and a "KRAB" domain (amino acid No. 2-97 of Kox1) of human Kox1 protein (NCBI protein database AAH24182; GI: 18848329). In one embodiment of the present invention, a Kid transcription repression domain was used, whose amino acid sequence and nucleic acid sequence encoding same are represented by SEQ ID NOs: 69 and 68, respectively.

The transcription activation or repression domains can be fused to the DNA binding domain to form a fusion protein.

Meanwhile, protein transduction domains result in uptake of the transduction domain and attached polypeptide into a cell. Representative PTDs useful in the present invention include a part of HIV TAT protein (e.g., amino acid region 47-57 of human HIV-1 virus Tat protein), a part of VP22 protein (e.g., HSV VP22 C-terminal 34 amino acid residues; Elliott and O'Hare *Cell* (1997) 88: 223-234 and U.S. Patent No. 6,184,038) or a part of an Antennapedia homeodomain (Derossi *et al.*, *J. Bio. Chem.* 269: 10444-10450).

Typically a PTD is linked to a zinc finger protein by producing the DNA binding domain of the zinc finger protein and the PTD as a single polypeptide chain, but other methods of for physically associating a PTD can be used. For example, the PTD can be associated by a non-covalent interaction (e.g., using biotin-avidin, coiled-coils, etc.) More typically, a PTD can be linked to a zinc finger protein, for example, using a flexible linker. Flexible linkers can include one or more glycine residues to allow for free rotation. For example, the PTD can be spaced from a DNA binding domain of the transcription factor by at least 10, 20, or 50 amino acids. A PTD can be located N- or C-terminal relative to a DNA binding domain.

The inventive polypeptides are useful to screen genes associated with

enhancing the production of monoclonal antibody or heterogeneous protein in a eukaryotic cell, preferably, in a mammalian cell, and they can up- or down-regulate the expression of the genes depending on the kinds of transcription regulation domains bound to the ZFD.

In addition, the present invention provides a transformed eukaryotic cell, preferably, a mammalian cell, which comprises the inventive polypeptide directly introduced into the cell or produced from a nucleic acid encoding same introduced into the cell. Non-limiting examples of the cells useful in the present invention include a CHO cell, a NS-O cell, a Per.C6 cell, a HEK293 cell and a HEK293T cell, wherein a CHO cell is preferred.

By using the transformed cell of the present invention, yield of the monoclonal antibody can be increased significantly under the same culture condition, as compared with the existing methods such as that using DHFR gene.

In one preferred embodiment of the present invention, the inventive polypeptide is selected from a library of ZFP-Kid artificial transcription factors prepared by fusing a transcription repression factor of mammalian cell, Kid, to be used as a zinc finger protein. There are two representative methods available for obtaining a zinc finger domain as a building unit of the library. One is about mutating a DNA contacting residue of existing zinc finger (Rebar E. J. & Pabo, C. O., *Science* (1994) 263: 671-673), and the other is about isolating a zinc finger domain from the genome of an organism (Bae K. –H. *et al.*, *Nature Biotechnol.*, (2003) 21: 275-280). Specific target base sequences of the zinc finger can be determined by phage display or the yeast one-hybrid method.

In one embodiment of the present invention, in order to transform a CHO cell with the zinc finger library in a high efficiency, a Kid domain and a $(ZFD)_4$ domain, which was obtained from existing zinc finger library, p3-$(ZFD)_4$ (WO 03/048345 and Park K. –S. *et al.*, *Nature Biotech*, (2003) 21: 1208-1214), are inserted into pLNCX2 vector, which was prepared by removing 2 *EcoR*I sites from a pLNCX retrovirus vector (Clontech, USA), to obtain a pLNCX2-$(ZFD)_4$-Kid library (Fig. 1).

A procedure of screening a zinc finger protein increasing the monoclonal antibody production from the library is shown in Fig. 2. Each DNA of the library is introduced together with pGag-Pol (Takara, Japan) and pVpack-VSV-G

(Stratagene, USA) to a HEK 293T cell for transformation, and the supernatant of the culture medium including virus particles packed with different ZFP artificial transcription factors are collected.

The supernatant is then added to the AKA cell, which was prepared by transforming a CHO cell with a gene of humanized antibody against TAG72 and amplifying in 20 nM MTX, to insert other genes of a ZFP-Kid artificial transcription factor into genome of the AKA cell in each well.

The increase of the monoclonal antibody production induced by a ZFP-Kid gene is compared with the antibody production induced by an AKA cell having ZFP-free pLNCX-Kid. Quantity of the monoclonal antibody is analyzed with ELISA using an antibody against human immunoglobulin, and ZFPs increasing the monoclonal antibody production in an AKA cell, i.e., ZFP LK33-Kid, LK35-Kid, LK50-Kid and LK52-Kid, are identified (Fig. 3 and Table 2).

ZFP LK52-Kid increases the monoclonal antibody production about 3 to 9 times than that of the Kid control group, depending on the culture period (Fig. 4). Further, when one amino acid at each of two zinc finger domains of LK52-Kid is mutated, no increase in the monoclonal antibody production is observed (Fig. 4). From these results, it is assumed that the inventive polypeptides control the transcription of intracellular genes, thereby controlling the expression of genes causing increase of the monoclonal antibody production.

In addition, the present invention provides a method of identifying the gene causing the increase of the monoclonal antibody production using the inventive polypeptide or nucleic acid encoding same. For example, a gene inducing a significant increase in the monoclonal antibody production can be identified as follows. A mammalian cell producing monoclonal antibody, e.g., a CHO cell transformed with a monoclonal antibody gene ("AKA cell"), is transformed with a zinc finger polypeptide comprising at least 2, preferably 2 to 6 zinc finger domains and a transcription repression domain connected thereto, or a nucleic acid encoding the zinc finger polypeptide. RNA is extracted from the transformed AKA cell, and cDNA probe is prepared by the reverse transcription. The probe is then subjected to reaction with a DNA chip having microarray of CHO cell genes to select a gene of which expression is repressed by ZFP, thereby causing the increase of the monoclonal antibody production.

In the present invention, the microarray can be conducted in accordance with a conventional method (Schena M. *et al.*, *Science* (1995) 270:467-470).

The inventive screening method using a ZFP library can also be applied to other animal cells (e.g., CHO, NS-O, Per.C6, HeLa, HEK293 and 293T cell) which is industrially used to produce a heterogeneous useful protein, for improving their useful characters (e.g., increase of protein production and prevention of apoptosis).

The following Examples are intended to further illustrate the present invention without limiting its scope.

Example 1: Construction of pLNCX-$(ZFD)_4$-Kid library

(Step 1) Preparation of a zinc finger protein expression library, p3-$(ZFD)_4$

A zinc finger library was prepared in accordance with the method of Bae, K. –H. *et al.* (*Nature Biotechnol.*, (2003) 21:275-280) using zinc finger domains of Table 1 which were separated from the human genome by PCR method or cloning and their specific target base sequences were determined by yeast one-hybrid method (Korean Patent Laid-open Publication No.10-2001-0084880; WO 01/60970; Bae, K. –H. *et al.*, *supra*), respectively.

Specifically, a HA-tag (SEQ ID NO: 16) and a nuclear localization signal (NLS, SEQ ID NO: 17) were inserted into the multiple cloning site of plasmid pCDNA3 (Invitrogen, USA), and the resulting vector was designated as plasmid p3 (Bae, K. –H. *et al.*, *supra*).

Table 1

Domain name	DNA sequence	Amino acid sequence	Target seq.* (5→3)
CSNR1	TATAAATGTAAGCAATGTGGGAAGGCTTTTGGATGTCCCTCAAAC CTTCGAAGGCATGGAAGGACTCAC (SEQ ID NO: 18)	YKCKQCGKAFGCPSNLRRHG RTH (SEQ ID NO: 19)	GAV
DSAR2	TACTCCTGTGGCATTTGTGGCAAATCCTTCTCTGACTCCAGTGCC AAAAGGAGACACTGCATTCTACAC (SEQ ID NO: 20)	YSCGICGKSFSDSSAKRRHC ILH (SEQ ID NO: 21)	RTC
DSCR	TACACATGCAGTGACTGTGGGAAGGCTTTCAGAGATAAATCATGT CTCAACAGACATCGGAGAACTCAT (SEQ ID NO: 22)	YTCSDCGKAFRDKSCLNRHR RTH (SEQ ID NO: 23)	GCC
DGNV	TTTCAATGTCGTATTTGTATGCGTAATTTTTCTGATTCTGGTAAT TTGCGTGTTCATATTCGTACTCAT (SEQ ID NO: 24)	FQCRICMRNFSDSGNLRVHI RTH (SEQ ID NO: 25)	AAC
DSNR	TATGCTTGCCCTGTCGAGTCCTGCGATCGCCGCTTTTCTGATTCG TCGAACCTTACCCGCCATATCCGCATCCAC (SEQ ID NO: 26)	YACPVESCDRRFSDSSNLTR HIRIH (SEQ ID NO: 27)	GAC
1SNR	TACAGGTGTAAGTACTGCGACCGCTCCTTCAGCATCTCTTCGAAC CTCCAGCGGCACGTCCGGAACATCCAC (SEQ ID NO: 28)	YRCKYCDRSFSISSNLQRHV RNIH (SEQ ID NO: 29)	GAW
QFNR	TATAAGTGTCATCAATGTGGGAAAGCCTTTATTCAATCCTTTAAC CTTCGAAGACATGAGAGAACTCAC (SEQ ID NO: 30)	YKCHQCGKAFIQSFNLRRHE RTH (SEQ ID NO: 31)	GAG
QSHV	TATGAGTGTGATCACTGTGGAAAATCCTTTAGCCAGAGCTCTCAT CTGAATGTGCACAAAAGAACTCAC (SEQ ID NO: 32)	YECDHCGKSFSQSSHLNVHK RTH (SEQ ID NO: 33)	HGA
QSNI	TACATGTGCAGTGAGTGTGGGCGAGGCTTCAGCCAGAAGTCAAAC CTCACCATACACCAGAGGACACAC (SEQ ID NO: 34)	YMCSECGRGFSQKSNLTIHQ RTH (SEQ ID NO: 35)	MAA
QSNK	TACAAGTGTGAAGAATGTGGCAAGGCTTTACCCAATCCTCAAAC CTTACTAAACATAAGAAAATTCAT (SEQ ID NO: 36)	YKCEECGKAFTQSSNLTKHK KIH (SEQ ID NO: 37)	DAA
QSNR1	TTTGAGTGTAAAGATTGCGGGAAGGCTTTCATTCAGAAGTCAAAC CTCATCAGACACCAGAGAACTCAC (SEQ ID NO: 38)	FECKDCGKAFIQKSNLIRHQ RTH (SEQ ID NO: 39)	GAA
QSNV2	TATGTTTGCTCAAAATGTGGGAAAGCCTTCACTCAGAGTTCAAAT CTGACTGTACATCAAAAAATCCAC (SEQ ID NO: 40)	YVCSKCGKAFTQSSNLTVHQ KIH (SEQ ID NO: 41)	NAA
QSSR1	TATAAGTGCCCTGATTGTGGGAAGAGTTTTAGTCAGAGTTCCAGC CTCATTCGCCACCAGCGGACACAC (SEQ ID NO: 42)	YKCPDCGKSFSQSSSLIRHQ RTH (SEQ ID NO: 43)	GYA
QTHQ	TATGAGTGTCACGATTGCGGAAAGTCCTTTAGGCAGAGCACCCAC CTCACTCAGCACCGGAGAATCCAC (SEQ ID NO: 44)	YECHDCGKSFRQSTHLTQHR RIH (SEQ ID NO: 45)	HGA
QTHR1	TATGAGTGTCACGATTGCGGAAAGTCCTTTAGGCAGAGCACCCAC CTCACTCGGCACCGGAGAATCCAC (SEQ ID NO: 46)	YECHDCGKSFRQSTHLTRHR RIH (SEQ ID NO: 47)	VGA
RDER1	TATGTATGCGATGTAGAGGGATGTACGTGGAAATTTGCCCGCTCA GATGAGCTCAACAGACACAAGAAAAGGCAC (SEQ ID NO: 48)	YVCDVEGCTWKFARSDELNR HKKRH (SEQ ID NO: 49)	GHG
RDHT	TTCCAGTGTAAAACTTGTCAGCGAAAGTTCTCCCGGTCCGACCAC CTGAAGACCCACACCAGGACTCAT (SEQ ID NO: 50)	FQCKTCQRKFSRSDHLKTHT RTH (SEQ ID NO: 51)	NGG
RDKR	TATGTATGCGATGTAGAGGGATGTACGTGGAAATTTGCCCGCTCA GATAAGCTCAACAGACACAAGAAAAGGCAC (SEQ ID NO: 52)	YVCDVEGCTWKFARSDKLNR HKKRH (SEQ ID NO: 53)	RGG
RDNQ	TTTGCCTGCCCTGAGTGTCCTAAGCGCTTCATGAGATCCGACAA CCTGACCCAGCATATCAAGACCCAC (SEQ ID NO: 54)	FACPECPKRFMRSDNLTQHI KTH (SEQ ID NO: 55)	AAG
RSHR	TATAAGTGCATGGAGTGTGGGAAGGCTTTTAACCGCAGGTCACAC CTCACACGGCACCAGCGGATTCAC (SEQ ID NO: 56)	YKCMECGKAFNRRSHLTRHQ RIH (SEQ ID NO: 57)	GGG
RSNR	TATATTTGCAGAAAGTGTGGACGGGGCTTTAGTCGGAAGTCCAAC CTTATCAGACATCAGAGGACACAC (SEQ ID NO: 58)	YICRKCGRGFSRKSNLIRHQ RTH (SEQ ID NO: 59)	GAG
VSNV	TATGAATGCGATCACTGTGGGAAAGCCTTCAGCGTCAGCTCCAAC CTGAACGTGCACAGAAGAATCCAC (SEQ ID NO: 60)	YECDHCGKAFSVSSNLNVHR RIH (SEQ ID NO: 61)	AAT
VSSR	TATACATGTAAACAGTGTGGGAAAGCCTTCAGTGTTTCCAGTTCC CTTCGAAGACATGAAACCACTCAC (SEQ ID NO: 62)	YTCKQCGKAFSVSSSLRRHE TTH (SEQ ID NO: 63)	GTD
VSTR	TATGAGTGTAATTACTGTGGAAAAACCTTTAGTGTGAGCTCAACC CTTATTAGACATCAGAGAATCCAC (SEQ ID NO: 64)	YECNYCGKTFSVSSTLIRHQ RIH (SEQ ID NO: 65)	GCW
WSNR	TACAGATGTGAGGAATGTGGCAAAGCCTTTAGGTGGCCCTCAAAC CTTACTAGACATAAGAGAATACAC (SEQ ID NO: 66)	YRCEECGKAFRWPSNLTRHK RIH (SEQ ID NO: 67)	GGW

* H: A or C; D: A or G or T; Y: C or T
N: A or C or G or T; W: A or T

Any one of DNA sequences encoding respective zinc finger domain listed in Table 1 was cloned into *EcoRI*/*NotI* restriction site of plasmid p3 to make plasmid p3-ZFD (*see* Fig. 5). Equal amounts of each plasmid p3 including any one of the 25 domains listed in Table 1 were collected to make a domain pool. A part of the pool was digested with *Xma*I and *Not*I restriction enzymes to obtain DNA inserts of each zinc finger domain. The other part of the pool was digested with *Age*I and *Not*I restriction enzymes to obtain long DNA fragments corresponding to vectors containing one zinc finger domain. The DNA inserts were cloned into the long DNA fragments to obtain a vector library expressing zinc finger proteins including two zinc finger domains. A part of the library was digested with *Age*I and *Not*I restriction enzymes to obtain DNA inserts including two zinc finger domains, and the DNA inserts were cloned into *AgeI*/*NotI* restriction sites of the library vectors including two zinc finger domains to obtain a library of vectors p3-$(ZFD)_4$ expressing zinc finger proteins including four zinc finger domains. A DNA encoding a zinc finger protein including four zinc finger domains can be separated from a vector of the library by digesting with *EcoR*I and *Not*I restriction enzymes.

(Step 2) Preparation of pLNCX2-$(ZFD)_4$-Kid library

A vector expressing a zinc finger protein fused with a transcription repression domain was prepared as follows (*see* Fig. 1).

First of all, in order to remove the *EcoR*I restriction site at nucleotide No. 1475 of retrovirus vector pLNCX (Clontech), site-directed mutagenesis was carried out using a mutagenesis kit (Stratagene, USA) and a pair of forward oligomer LNCX1-F (SEQ ID NO: 70, 5′ -TAGGCGCCGGAATTtCGATCTGATCAAGA-3′ ; hereinafter, a small letter represents a mutated base) and reverse oligomer LNCX1-R (SEQ ID NO: 71, 5′ -TCTTGATCAGATCGaAATTCCGGCGCCTA-3′), thereby obtaining plasmid pLNCX1. The plasmid pLNCX1 was digested with *EcoR*I, and then treated with T4 polymerase to make blunt ends, which were then self-ligated to remove two *EcoR*I restriction sites therefrom to produce plasmid pLNCX2.

A transcription repression domain was prepared as follows. To amplify Kid domain of a rat, PCR was carried out using plasmid pTet-tTS (Clontech) being used as a template and a pair of forward oligomer Kid-F (SEQ ID NO: 72, 5′ -GGGCGGCCGCTAAATTCGTGTCAGTGACA-3′) and reverse oligomer Kid-R1 (SEQ ID NO: 73, 5′ -CCGCTCGAGTTACCAGGGATCCTCTCC-3′). The resulting product was digested with *Not*I and *Xho*I restriction enzymes and inserted into *Not*I/*Xho*I-digested plasmid pLFD-p65 (Bae, K. –H. *et al.*, *Nature Biotechnol.*, *supra*) to obtain a plasmid pLFD-Kid. The plasmid pLFD-Kid was sequentially treated with *Apa*I and T4 polymerase to have blunt ends, and then treated with *Hind*III to obtain a Kid fragment.

The plasmid pLNCX2 was sequentially treated with *Cla*I and T4 polymerase to have blunt ends, and then digested with *Hind*III. The Kid fragment obtained above was inserted thereto to produce plasmid pLNCX2-Kid (*see* Fig. 6). In Fig.6, (*Apa*I/*Cla*I) represents that the two restriction enzyme sites were removed by the above procedure.

The p3-$(ZFD)_4$ library prepared in (Step 1) was digested with *Eco*RI and *Not*I to obtain $(ZFD)_4$ DNA fragments, and the fragments were inserted into a plasmid pLNCX2-Kid digested with *Eco*RI and *Not*I to obtain a plasmid pLNCX2-$(ZFD)_4$-Kid (*see* Fig. 7). In Fig.7, each of F1, F2, F3 and F4 represents one zinc finger domain, and (*Apa*I/*Cla*I) has the same meaning as defined in Fig. 6.

In order to prepare a library, *E. coli* cells were transformed with pLNCX2-$(ZFD)_4$-Kid vectors, and library plasmids were isolated from more than 5,000 colonies of *E. coli* using an Accuprep plasmid extraction kit (Bioneer, Korea).

Example 2: Screening of ZFP increasing the production of monoclonal antibody

(Step 1) Production of ZFP-containing virus in 293T cell

6×10^4 of 293T cells (ATCC CRL-11268) were put into each well of a 96-

well culture plate provided with DMEM (JBI, Korea) containing heat-treated 10 % FBS (JBI, Korea), and cultured for a day at 37 ℃ in a CO_2 incubator.

Transformation was conducted as follows. First of all, 100 ng of plasmid pLNCX2-$(ZFD)_4$-Kid prepared in Example 1 or ZFP-free pLNCX2-Kid control group vector, 50 ng of pGag-Pol virus vector (Takara, Japan) and 50 ng of plasmid pVpack-VSV-G (Stratagene, USA) were mixed with 25 ㎕ of Opti-MEM (Invitrogen, USA) (Mixture A). Aside from this, 0.5 ㎕ of Lipofectamine 2000 (Invitrogen) was mixed with 25 ㎕ of Opti-MEM (Mixture B). Mixture B was placed at room temperature for 5 minutes, and mixed with mixture A. The resulting mixture was placed at room temperature for 20 minutes (Mixture C). The medium was removed from each well of the above culture plate, and mixture C was added thereto to transform 293T cells. After 10-hour culture, the medium was replaced with DMEM containing 10 mM sodium butyrate and 10 % FBS (JBI, Korea) to increase the virus production. Further, after 12-hour culture, the medium was replaced with DMEM including 10 % FBS (JBI, Korea). The cells were cultured in a CO_2 incubator for 24 hours and a supernatant including virus was collected from the culture.

(Step 2) Transduction of AKA cell using ZFP-containing virus

In order to insert and screen a zinc finger library, an AKA cell (obtained from Dr. Hyo-Jeong Hong of Korea Research Institute of Bioscience and Biotechnology) was used. AKA cell was prepared by transforming a CHO cell with a gene of humanized antibody against TAG72 glycoprotein (Korea Patent Laid-open Publication No. 2000-0005885) and amplified using 20 nM MTX, in accordance with a conventional method (Kim, S. J., *et al.*, *Biotechnol&Bioeng.* (1998) 58: 73-84).

One day before collecting the supernatant in (Step 1), 1.5 X 10^4 of AKA cells were put into each well of a 96-well culture plate containing alpha-MEM (JBI, Korea) containing dialyzed 10 % FBS (JBI, Korea), and cultured for a day at 37 ℃ in a CO_2 incubator. 25 ㎕ of the supernatant of (Step 1) was mixed with 25 ㎕ of alpha-MEM containing 10 % dialyzed FBS and 0.05 ㎕ of polybrene (80 ㎍/㎖), and the resulting mixture was added to each well having cultured

AKA cells to transform the AKA cells. After 24 hours, the medium was replaced with fresh alpha-MEM and the cells were further incubated for 24 hours. 50 ㎕ of the supernatant was taken from the transformed AKA cell culture and frozen.

(Step 3) ELISA for screening of ZFP increasing the monoclonal antibody production

50 ㎕ of 0.05 M sodium carbonate (pH 9.6) containing 0.5 ㎍ of anti-mouse IgG (Sigma) was put into each well of a 96-well immuno plate (Nunc, Denmark) and the plate was stored in a 37 ℃ incubator for 2 hours. Then the plate was washed three times with distilled water, and 50 ㎕ of diluted supernatant of (Step 2), which was 100 times diluted with PBS (0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride, pH 7.4) containing 0.25 % BSA and 0.05 % Tween-20, was added to each well of the plate. After 2 hours at room temperature, the plate was washed three times with distilled water, and 50 ㎕ of diluted anti-human IgG-peroxide conjugate (Sigma), which was 10,000 times diluted with PBS containing 0.25 % BSA, and 0.05 % Tween-20, was added to each well of the plate. After 1 hour, the plate was washed three times with distilled water. 70 ㎕ of TMB solution (Sigma) was added to each well of the plate, and the reaction was carried out for 10 minutes. The absorbance of the wells was measured at 655 nm by powerwave 340 (Bio-TEK Instruments, INC, USA). As a result, most of the wells showed similar absorbance to that of ZFP-free pLNCX2-Kid control group vector. However, four pLNCX2-$(ZFD)_4$-Kid plasmids showed higher absorbance than that of the control group and they were subjected to further experiment.

Example 3: Change of the monoclonal antibody production by four ZFPs in AKA cell

The antibody productions of four ZFP's were shown to be higher than that of the control group with pLNCX2-Kid inserted therein. For confirmation, Example 2 was repeated, except that length of time the reaction mixture was

placed at room temperature has changed.

Lipofectamine 2000 was mixed with Opti-MEM and placed at room temperature for 20 minutes. The mixture was mixed with Opti-MEM including plasmids pLNCX2-ZFP-Kid, pGag-Pol and pVpack-VSV-G and then was placed at room temperature for 40 minutes. 293T cells cultured in a 96-well plate were transformed with the mixture, each plasmid of pLNCX2 and pLNCX2-Kid being used as control plasmid.

AKA cells were transformed with virus in accordance with the procedure of (Step 2) of Example 2, and the medium was replaced with new medium after 24 hours and cultured for 24 hours. Each supernatant including antibody was collected and then diluted 250 and 500 times. Next, they were subjected to ELISA. As can be seen in Fig. 3, four ZFP-Kids, i.e., LK33-Kid, LK35-Kid, LK50-Kid and LK52-Kid showed about 2 to 6 times increase in the production of antibody in AKA cells. Zinc finger domains and target sequences of these ZFPs are listed in Table 2.

Table 2

ZFP name	F1	F2	F3	F4	Target sequence
LK33	RDHT	QTHR1	QSNK	QSSR1	GYADAAVGANGG (SEQ ID NO: 74)
LK35	RDHT	QSNR1	QTHR1	QSNI	MAAVGAGAANGG (SEQ ID NO: 75)
LK50	QTHR1	RDKR	RDHT	QTHR1	VGANGGRGGVGA (SEQ ID NO: 76)
LK52	RDHT	QSHV	QTHQ	QSSR1	GYAHGAHGANGG (SEQ ID NO: 77)
Y: C or T; D: A or G or T; V: A or G or C; M: A or C; N: A or G or C or T; R: A or G; H: A or C or T					

Example 4: Change in the monoclonal antibody production by LK52 in AKA cell in accordance with length of time

(Step 1) Production of ZFP-containing virus in 293T cell

5×10^5 293T cells (ATCC CRL-11268) were put into each well of a 24-

well culture plate provided with DMEM (JBI, Korea) containing heat-treated 10 % FBS (JBI, Korea), and cultured for 1 day at 37 ℃ in a CO_2 incubator.

400 ng of plasmid pLNCX2-LK52-Kid, 200 ng of pGag-Pol virus vector (Takara, Japan) and 200 ng of plasmid pVpack-VSV-G (Stratagene, USA) were mixed with 50 ㎕ of Opti-MEM (Invitrogen, USA) (Mixture A). Aside from this, 2 ㎕ of Lipofectamine 2000 (Invitrogen) was mixed with 50 ㎕ of Opti-MEM (Mixture B). Mixture B was placed at room temperature for 20 minutes before being mixed with mixture A. The resulting mixture was placed at room temperature for 40 minutes (Mixture C). The medium was removed from each well of the above culture plate, and mixture C was added thereto to transform 293T cells. After 10-hour culture, the medium were replaced with DMEM containing 10 mM sodium butyrate and 10 % FBS (JBI, Korea) to increase the production of virus. Further, after 12 hour culture, the medium was replaced with fresh DMEM containing 10 % FBS (JBI, Korea). The cells were cultured in a CO_2 incubator for 24 hours and a supernatant of transformed 293T cells including virus was collected from the culture.

(Step 2) Transduction of AKA cell using ZFP-containing virus

One day before collecting the supernatant in (Step 1), 3 X 10^4 AKA cells were put into each well of a 24-well culture plate containing alpha-MEM (JBI, Korea) containing dialyzed 10 % FBS (JBI, Korea), and cultured for a day at 37 ℃ in a CO_2 incubator. 125 ㎕ of the supernatant of (Step 1) was mixed with 125 ㎕ of alpha-MEM containing 10 % dialyzed FBS and 0.25 ㎕ of polybrene (80 ㎍/㎖), and the resulting mixture was added to each well having cultured AKA cells to transform the AKA cells. After 24 hours, the medium was replaced with new alpha-MEM containing 10 % FBS and the cells were further incubated for 24, 48, 72, 96 and 120 hours (i.e., 3, 4, 5, 6 and 7 days shown in Fig. 4). 200 ㎕ of each supernatant after transformation, 200 ㎕ of each supernatant before transformation (1 day shown in Fig. 4), and 200 ㎕ of each supernatant after transformation but before replacement of medium (2 day shown in Fig. 4) were taken from the transformed AKA cell cultures and frozen.

(Step 3) ELSIA for screening of ZFP increasing the monoclonal antibody production

The stored supernatants of (Step 2) were subjected to ELISA in accordance with the same procedure of (Step 3) of Example 2.

As shown in Fig. 4, with the increase in culture time, the monoclonal antibody production of LK52-Kid increased to as high as 37 ㎍/㎖, 9 times higher when compared with the control group.

Example 5: Activity of mLK52-Kid transduced with mutant ZFP

To examine whether the ZFP is a direct cause of the increase in the monoclonal antibody production, both arginine residues at DNA contacting residue –1 of the first finger RDHT and arginine residues at DNA contacting residue 6 of the fourth finger QSSR1 of LK52-Kid were mutated to alanines to change the DNA binding site of LK52. The mutagenesis was conducted as shown in Fig. 8.

First of all, PCRs were carried out by using plasmid pLNCX2-LK52-Kid as a template and a respective oligomer pairs of ① NLS-F (SEQ ID NO: 78, 5′ -CCTCCAAAAAAGAAGAGAAAGGTA-3′) and mutRDHT-R (SEQ ID NO: 79, 5′ -CAGGTGGTCGGAggcGGAGAACTTTCG-3′), ② mutRDHT-F (SEQ ID NO: 80, 5′ -CGAAAGTTCTCCgccTCCGACCACCTG-3′) and QTHQ-R (SEQ ID NO: 81, 5′ -CCTAAAGGACTTTCCGCAATCGTGACACTC-3′), ③ QTHQ-F (SEQ ID NO: 82, 5′ -GAGTGTCACGATTGCGGAAAGTCCTTTAGG-3′) and mutQSSR1-R (SEQ ID NO: 83, 5′ -GTGTCCGCTGGTGGgcAATGAGGCTGGAAC-3′) and ④ mutQSSR1-F (SEQ ID NO: 84, 5′ -GTTCCAGCCTCATTgcCCACCAGCGGACAC-3′) and Kid-R2 (SEQ ID NO: 85, 5′ -CCGCTCGAGCCAGGGGTCCTCTCC-3′) to obtain the first products. Further, the 2nd PCRs were carried out by employing the first products of ① and ② as templates and an oligomer pair of NLS-F (SEQ ID NO: 78) and QTHQ-R (SEQ ID NO: 81) (⑤); and by

employing the first products of ③ and ④ as templates and an oligomer pair of QTHQ-F (SEQ ID NO: 82) and Kid-R2 (SEQ ID NO: 85) (⑥), respectively. Finally, a 3rd PCR was carried out by employing the products of ⑤ and ⑥ as templates and an oligomer pair of NLS-F (SEQ ID NO: 78) and Kid-R2 (SEQ ID NO: 85) to obtain a DNA product having desired mutation. The final PCR product was digested with *EcoR*I and *Not*I to obtain mutated mLK52 DNA fragment. The DNA fragment was cloned into the *EcoRI*/*Not*I restriction site of plasmid pLNCX2-LK52-Kid, wherein wild type LK52 gene was removed. *E. coli* DH5 α cells were transformed with the resulting plasmid. Plasmids were isolated from colonies thus obtained and sequenced to obtain a plasmid pLNCX2-mLK52-Kid including a mutated mLK52 gene.

AKA cells were transformed with this mutated pLNCX2-mLK52-Kid using virus as described in Example 4, and increase in the monoclonal antibody production by the ZFP was examined. As shown in Fig. 4, an AKA cell including mLK52-Kid showed similar monoclonal antibody production to that of a ZFP-free control cell expressing Kid only. Considering that AKA cell transformed with LK52-Kid showed about 9 times increase of the production, this result demonstrate that specific DNA binding ability of LK52 is responsible for the increase of the monoclonal antibody production.

Example 6: Change of the monoclonal antibody production by LK52 in various cells

In order to examine whether LK33-Kid, LK35-Kid, LK50-Kid and LK52-Kid also induce an increase in the monoclonal antibody production in other monoclonal antibody-producing cells than AKA cell, the ZFP-Kids were introduced into SH2-0.32 and ISU-ABC cells (obtained from Professor Gyun-Min LEE of Korea Advanced Institute of Science and Technology), which are CHO cells producing monoclonal antibody, and the amount of produced monoclonal antibody was examined. SH2-0.32 cell was prepared by transforming a CHO cell with a gene for a monoclonal antibody against the surface antigen of Hepatitis B virus and amplifying the gene to increase the

monoclonal antibody production (Kim, N. S. *et al.*, *Biotech&Bioeng.* (2001) 71:184-193). ISU-ABC cell was prepared by transforming CHO cell with a gene for a monoclonal antibody against the glycoprotein receptor of human blood platelet and amplifying the gene to increase the monoclonal antibody production. The amplification of a specific gene in CHO cell was carried out in accordance with conventional methods such as gene amplification using DHFR (dihydrofolate reductase) gene and MTX (Kim, S. J. *et al.*, *Biotechnol&Bioeng.* (1998) 58: 73-84).

The examination was conducted in accordance with the same procedure as described in Example 2, except that the medium was replaced with 100 ㎕ of new one 1 day after the transformation with virus; 30 ㎕ of the supernatant was collected after 1 day; the residual supernatant was collected after 3 days; and the concentration of antibody secreted into the medium was determined by using the supernatants. The average and standard deviation were calculated using 3-4 wells per each plasmid sample, and the ELISA was conducted as follows.

100 ㎕ of 0.05 M sodium carbonate (pH 9.6) including 0.2 ㎍ of anti-mouse IgG (Sigma) was put into each well of a 96-well immuno plate (Nunc, Denmark) and placed in a 37 ℃ incubator for 2 hours. The plate was washed three times with PBST (PBS including 0.05 % Tween-20). 300 ㎕/well of 2 % BSA was added to the wells and the plate was placed at 37 ℃ for 2 hours to block the non-specific reactions, and washed three times with PBST. 100 ㎕ of diluted supernatant, which was prepared by diluting 1,000 times the supernatants obtained above with PBS containing 0.25 % BSA and 0.05 % Tween-20, was added to each well of the plate. After 2 hours at room temperature, the plate was washed three times with PBST, and 100 ㎕ of diluted anti-human IgG-HRP conjugate (Sigma), which was 2,000 times diluted with PBS containing 0.25 % BSA and 0.05 % Tween-20, was added to each well of the plate. After 1 hour, the plate was washed three times with distilled water. 100 ㎕ of substrate mixture (mixture of 10 ㎖ of PC buffer [2.55 g of $C_6H_8O_7 \cdot H_2O$ and 3.65 g of Na_2HPO_4 in 500 ㎖ of distilled water, pH 5.0], 12 ㎕ of O-phenylenediamine and 4 ㎕ of H_2O_2) was added to each well of the plate, and left there for reaction for 2-3 minutes. The reaction was terminated by adding 50 ㎕ of 1 M H_2SO_4

to each well. The result was detected by ELISA reader (Bio-rad, model-680) at 490 nm, and shown in Tables 3 and 4.

Table 3: Monoclonal antibody production in SH2-0.32 cell

Kind of ZFP	Monoclonal antibody production after 1 day		Monoclonal antibody production after 3 days	
	Average (±S.D.) (μg/mℓ)	Fold	Average (±S.D.) (μg/mℓ)	Fold
LNCX2	3.08 (±0.21)	1.00	8.69 (±0.52)	1.00
Kid	2.83 (±0.33)	0.92	7.40 (±1.03)	0.85
LK33-Kid	5.67 (±0.38)	1.84	32.25 (±3.48)	3.71
LK35-Kid	5.15 (±0.38)	1.67	21.76 (±2.14)	2.50
LK50-Kid	4.40 (±0.87)	1.43	23.93 (±2.86)	2.75
LK52-Kid	5.92 (±0.89)	1.92	36.28 (±3.00)	4.17

Table 4: Monoclonal antibody production in ISU-ABC cell

Kind of ZFP	Monoclonal antibody production after 1 day		Monoclonal antibody production after 3 days	
	Average (±S.D.) (μg/mℓ)	Fold	Average (±S.D.) (μg/mℓ)	Fold
LNCX2	17.31 (±1.87)	1.00	79.43 (±10.64)	1.00
Kid	15.33 (±2.09)	0.89	77.60 (±9.07)	0.98
LK33-Kid	18.58 (±1.91)	1.07	103.22 (±15.63)	1.30
LK35-Kid	19.42 (±1.31)	1.12	103.45 (±9.97)	1.30
LK50-Kid	17.44(±0.65)	1.01	120.04 (±14.51)	1.51
LK52-Kid	20.45 (±3.06)	1.18	143.07 (±18.02)	1.80

As can be seen in Tables 3 and 4, LK33-Kid, LK35-Kid, LK50-Kid and LK52-Kid increased the monoclonal antibody productions after 3 days in SH2-0.32 and ISU-ABC cells by 2.5 to 4.1 folds and by 1.3 to 1.8 folds, respectively, and LK52-Kid showed the best result. This result indicates that ZFP screened in an AKA cell worked similarly in CHO cells producing other kind of antibody. Accordingly, it is expected that ZFP screened in one cell may have similar effect in other homogeneous cells having a similar function.

Example 7: Real time PCR

In order to examine the mechanisms of LK33-Kid, LK35-Kid, LK50-Kid and LK52-Kid increasing the monoclonal antibody production in an AKA cell, mRNAs of monoclonal antibody of AKA cells expressing the 4 ZFPs were quantified by real time PCR.

Viruses having LK33-Kid, LK35-Kid, LK50-Kid and LK52-Kid were produced and transformed into AKA cells by the method described in Example 3. The plasmid pLNCX2-Kid was used as a control. The media was replaced after 24 hours and then the cells were collected after 2 days. RNA was extracted from the cells using Trizol (Invitrogen). Reverse transcription reaction was conducted using 1 μg of the extracted RNA and SuperScript II kit (Invitrogen). 8 μl of the product was mixed with 10 μl of 2X SYBR (Qiagen), and the mixture was subjected to real time PCR (Rotor-Gene 2000, Corbett research, Australia) using an oligomer pair of 1 μl of AKA-F (SEQ ID NO: 86, 5′ -GATGGGCCCTTGGTGCTGGCT-3′) and 1 μl of AKA-R (SEQ ID NO: 87, 5′ -GACGAATTCACTCTAACCATGGAA-3′), or a pair of 1 μl of GAPDH-F (SEQ ID NO: 88, 5′ -CCGAGTATGTTGTGGAATCTACTG-3′) and 1 μl of GAPDH-R (SEQ ID NO: 89, 5′ -GACAATCTTGAGGGAGTTGTCATA-3′). The concentration of each oligomer was 10 pmol/μl. As can be seen in Fig. 9, all ZFPs, i.e., LK33-Kid, LK35-Kid, LK50-Kid and LK52-Kid, increased the amount of mRNA of monoclonal antibody 3-6 time than that of GAPDH mRNA.

In addition, the ZFPs may also act on the genes of proteins related to the translation or secretion of a monoclonal antibody.

While the invention has been described with respect to the above specific embodiments, it should be recognized that various modifications and changes may be made and also fall within the scope of the invention as defined by the claims that follow.

What is claimed is:

1. A polypeptide capable of increasing the amount of a monoclonal antibody produced by a eukaryotic cell relative to a cell that does not include the polypeptide, wherein the polypeptide comprises a DNA binding domain that includes a plurality of zinc finger domains.

2. The polypeptide of claim 1, wherein the DNA binding domain comprises at least 2 zinc finger domains selected from the group consisting of the following amino acid sequences:

$CX_{(2-5)}CXXXBXRXXHJXTHX_{(3-5)}H$ (SEQ ID NO: 2);
$CX_{(2-5)}CXXXBXQXXHJXRHX_{(3-5)}H$ (SEQ ID NO: 3);
$CX_{(2-5)}CXXXBXQXXNJXKHX_{(3-5)}H$ (SEQ ID NO: 4);
$CX_{(2-5)}CXXXBXQXXSJXRHX_{(3-5)}H$ (SEQ ID NO: 5);
$CX_{(2-5)}CXXXBXQXXNJXRHX_{(3-5)}H$ (SEQ ID NO: 6);
$CX_{(2-5)}CXXXBXQXXNJXIHX_{(3-5)}H$ (SEQ ID NO: 7);
$CX_{(2-5)}CXXXBXRXXKJXRHX_{(3-5)}H$ (SEQ ID NO: 8);
$CX_{(2-5)}CXXXBXQXXHJXVHX_{(3-5)}H$ (SEQ ID NO: 9); and
$CX_{(2-5)}CXXXBXQXXHJXQHX_{(3-5)}H$ (SEQ ID NO: 10);

where B is phenylalanine or tyrosine; J is a hydrophobic amino acid; and X is any amino acid.

3. The polypeptide of claim 1, wherein the DNA binding domain includes, in N-terminal to C-terminal order, the first, second, third and fourth zinc finger domains, wherein

(1) the DNA contacting residues at positions –1, 3 and 6 of the first zinc finger domain are R, H and T, respectively; those of the second zinc finger domain are Q, H and R, respectively; those of the third zinc finger domain are Q, N and K, respectively; and those of the fourth zinc finger domain are Q, S and R, respectively;

(2) the DNA contacting residues at positions –1, 3 and 6 of the first zinc finger domain are R, H and T, respectively; those of the second zinc finger domain are Q, N and R, respectively; those of the third zinc finger domain are

Q, H and R, respectively; and those of the fourth zinc finger domain are Q, N and I, respectively;

(3) the DNA contacting residues at positions –1, 3 and 6 of the first zinc finger domain are Q, H and R, respectively; those of the second zinc finger domain are R, K and R, respectively; those of the third zinc finger domain are R, H and T, respectively; and those of the fourth zinc finger domain are Q, H and R, respectively; or

(4) the DNA contacting residues at positions –1, 3 and 6 of the first zinc finger domain are R, H and T, respectively; those of the second zinc finger domain are Q, H and V, respectively; those of the third zinc finger domain are Q, H and Q, respectively; and those of the fourth zinc finger domain are Q, S and R, respectively.

4. The polypeptide of claim 3, wherein the DNA binding domain has the amino acid sequence of any one of SEQ ID NOs: 12 to 15.

5. The polypeptide of claim 1, which further comprising a domain selected form the group consisting of a transcription activation domain, a transcription repression domain, a protein transduction domain and a combination thereof connected to the DNA binding domain.

6. The polypeptide of claim 5, wherein the transcription activation domain is selected from the group consisting of a Gal4 activation domain of yeast, a VP16 domain of herpes simplex virus and a p65 domain of a mammalian cell.

7. The polypeptide of claim 5, wherein the transcription repression domain is Kid or KOX repression domain.

8. The polypeptide of claim 5, wherein the protein transduction domain is a part of a TAT protein, a VP22 protein or an Antennapedia homeodomain.

9. The polypeptide of claim 1, wherein the eukaryotic cell is a

mammalian cell.

10. The polypeptide of claim 9, wherein the mammalian cell is selected from the group consisting of a CHO (Chinese Hamster Ovary) cell, a HEK293T cell, a HEK293 cell, a PerC6 cell and a NS-O cell.

11. A nucleic acid that comprises a sequence encoding the polypeptide of claim 1.

12. The nucleic acid of claim 11, wherein the polypeptide comprises a DNA binding domain that includes at least 2 zinc finger domains selected from the group consisting of the following amino acid sequences:

$CX_{(2-5)}CXXXBXRXXHJXTHX_{(3-5)}H$ (SEQ ID NO: 2);
$CX_{(2-5)}CXXXBXQXXHJXRHX_{(3-5)}H$ (SEQ ID NO: 3);
$CX_{(2-5)}CXXXBXQXXNJXKHX_{(3-5)}H$ (SEQ ID NO: 4);
$CX_{(2-5)}CXXXBXQXXSJXRHX_{(3-5)}H$ (SEQ ID NO: 5);
$CX_{(2-5)}CXXXBXQXXNJXRHX_{(3-5)}H$ (SEQ ID NO: 6);
$CX_{(2-5)}CXXXBXQXXNJXIHX_{(3-5)}H$ (SEQ ID NO: 7);
$CX_{(2-5)}CXXXBXRXXKJXRHX_{(3-5)}H$ (SEQ ID NO: 8);
$CX_{(2-5)}CXXXBXQXXHJXVHX_{(3-5)}H$ (SEQ ID NO: 9); and
$CX_{(2-5)}CXXXBXQXXHJXQHX_{(3-5)}H$ (SEQ ID NO: 10);

where B is phenylalanine or tyrosine; J is a hydrophobic amino acid; and X is any amino acid.

13. The nucleic acid of claim 11, wherein the polypeptide comprises a DNA binding domain that includes, in N-terminal to C-terminal order, the first, second, third and fourth zinc finger domains, wherein

(1) the DNA contacting residues at positions –1, 3 and 6 of the first zinc finger domain are R, H and T, respectively; those of the second zinc finger domain are Q, H and R, respectively; those of the third zinc finger domain are Q, N and K, respectively; and those of the fourth zinc finger domain are Q, S and R, respectively;

(2) the DNA contacting residues at positions –1, 3 and 6 of the first zinc

finger domain are R, H and T, respectively; those of the second zinc finger domain are Q, N and R, respectively; those of the third zinc finger domain are Q, H and R, respectively; and those of the fourth zinc finger domain are Q, N and I, respectively;

(3) the DNA contacting residues at positions –1, 3 and 6 of the first zinc finger domain are Q, H and R, respectively; those of the second zinc finger domain are R, K and R, respectively; those of the third zinc finger domain are R, H and T, respectively; and those of the fourth zinc finger domain are Q, H and R, respectively; or

(4) the DNA contacting residues at positions –1, 3 and 6 of the first zinc finger domain are R, H and T, respectively; those of the second zinc finger domain are Q, H and V, respectively; those of the third zinc finger domain are Q, H and Q, respectively; and those of the fourth zinc finger domain are Q, S and R, respectively.

14. The nucleic acid of claim 11, wherein the polypeptide further comprises a transcription activation domain, a transcription repression domain, or a protein transduction domain connected to the DNA binding domain.

15. A eukaryotic cell containing a gene encoding a monoclonal antibody and the nucleic acid that comprises a sequence encoding the polypeptide of claim 1.

16. The eukaryotic cell of claim 15, which is a mammalian cell.

17. The eukaryotic cell of claim 15, which is selected from the group consisting of a CHO cell, a HEK293T cell, a HEK293 cell, a PerC6 cell and a NS-O cell.

18. The eukaryotic cell of claim 15, wherein the polypeptide comprises a DNA binding domain that includes at least 2 zinc finger domains selected from the group consisting of the following amino acid sequences:

$CX_{(2-5)}CXXXBXRXXHJXTHX_{(3-5)}H$ (SEQ ID NO: 2);

$CX_{(2-5)}CXXXBXQXXHJXRHX_{(3-5)}H$ (SEQ ID NO: 3);
$CX_{(2-5)}CXXXBXQXXNJXKHX_{(3-5)}H$ (SEQ ID NO: 4);
$CX_{(2-5)}CXXXBXQXXSJXRHX_{(3-5)}H$ (SEQ ID NO: 5);
$CX_{(2-5)}CXXXBXQXXNJXRHX_{(3-5)}H$ (SEQ ID NO: 6);
$CX_{(2-5)}CXXXBXQXXNJXIHX_{(3-5)}H$ (SEQ ID NO: 7);
$CX_{(2-5)}CXXXBXRXXKJXRHX_{(3-5)}H$ (SEQ ID NO: 8);
$CX_{(2-5)}CXXXBXQXXHJXVHX_{(3-5)}H$ (SEQ ID NO: 9); and
$CX_{(2-5)}CXXXBXQXXHJXQHX_{(3-5)}H$ (SEQ ID NO: 10);
where B is phenylalanine or tyrosine; J is a hydrophobic amino acid; and X is any amino acid.

19. The eukaryotic cell of claim 15, wherein the polypeptide comprises a DNA binding domain that includes, in N-terminal to C-terminal order, the first, second, third and fourth zinc finger domains, wherein

(1) the DNA contacting residues at positions –1, 3 and 6 of the first zinc finger domain are R, H and T, respectively; those of the second zinc finger domain are Q, H and R, respectively; those of the third zinc finger domain are Q, N and K, respectively; and those of the fourth zinc finger domain are Q, S and R, respectively;

(2) the DNA contacting residues at positions –1, 3 and 6 of the first zinc finger domain are R, H and T, respectively; those of the second zinc finger domain are Q, N and R, respectively; those of the third zinc finger domain are Q, H and R, respectively; and those of the fourth zinc finger domain are Q, N and I, respectively;

(3) the DNA contacting residues at positions –1, 3 and 6 of the first zinc finger domain are Q, H and R, respectively; those of the second zinc finger domain are R, K and R, respectively; those of the third zinc finger domain are R, H and T, respectively; and those of the fourth zinc finger domain are Q, H and R, respectively; or

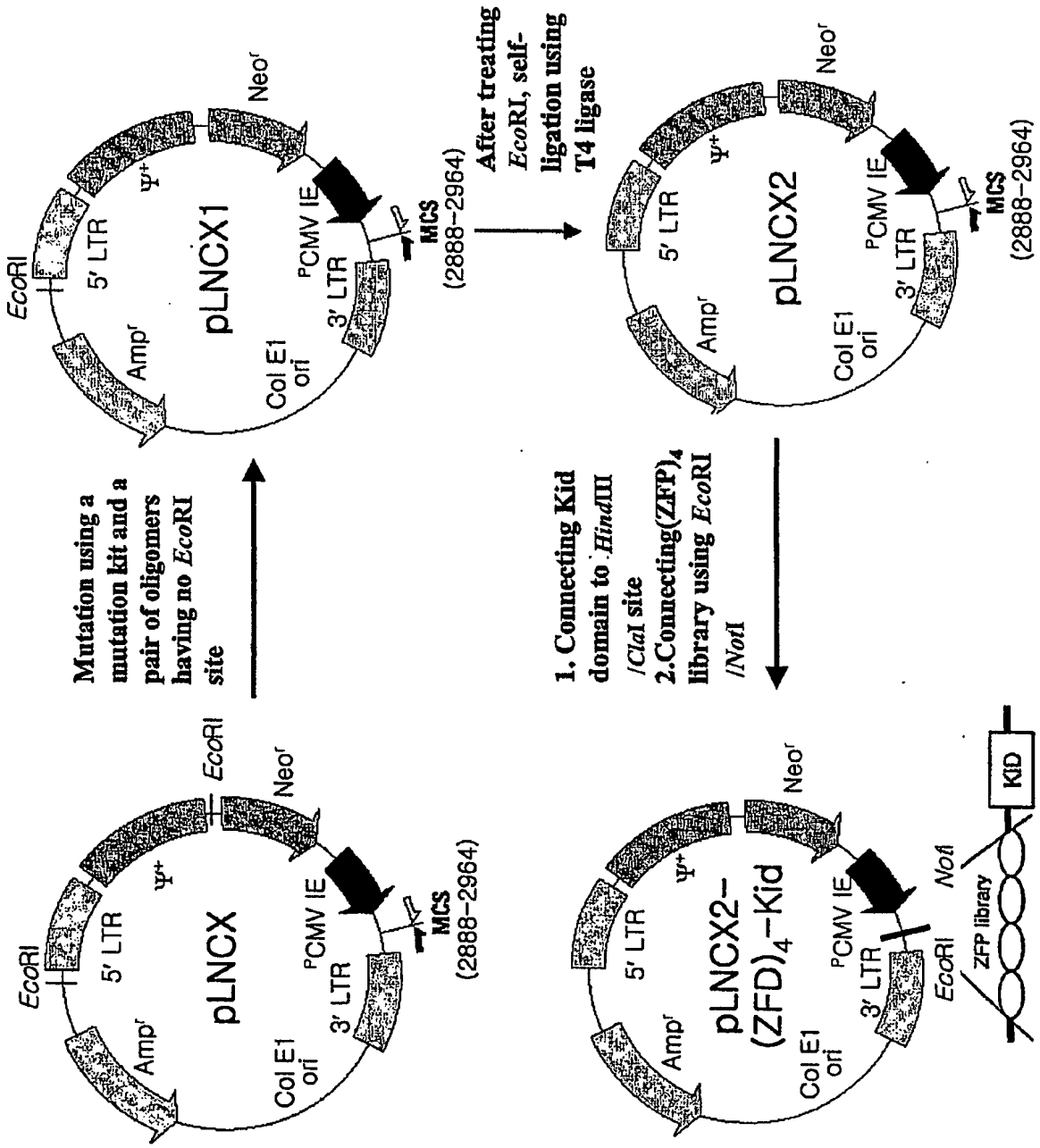
(4) the DNA contacting residues at positions –1, 3 and 6 of the first zinc finger domain are R, H and T, respectively; those of the second zinc finger domain are Q, H and V, respectively; those of the third zinc finger domain are Q, H and Q, respectively; and those of the fourth zinc finger domain are Q, S and

R, respectively.

20. The eukaryotic cell of claim 15, wherein the polypeptide further comprises a transcription activation domain, a transcription repression domain, or a protein transduction domain connected to the DNA binding domain.

21. A method for increasing the monoclonal antibody production in a eukaryotic cell, which comprises the step of introducing the polypeptide of claim 1 or the nucleic acid of claim 11 into the cell.

Fig. 1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EcoRI
NotI
Neor
Ampr
Ψ+
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3' LTR
PCMV IE
MCS
(2888-2964)
Col E1 ori
ZFP library
KID
Mutation using a mutation kit and a pair of oligomers having no EcoRI site
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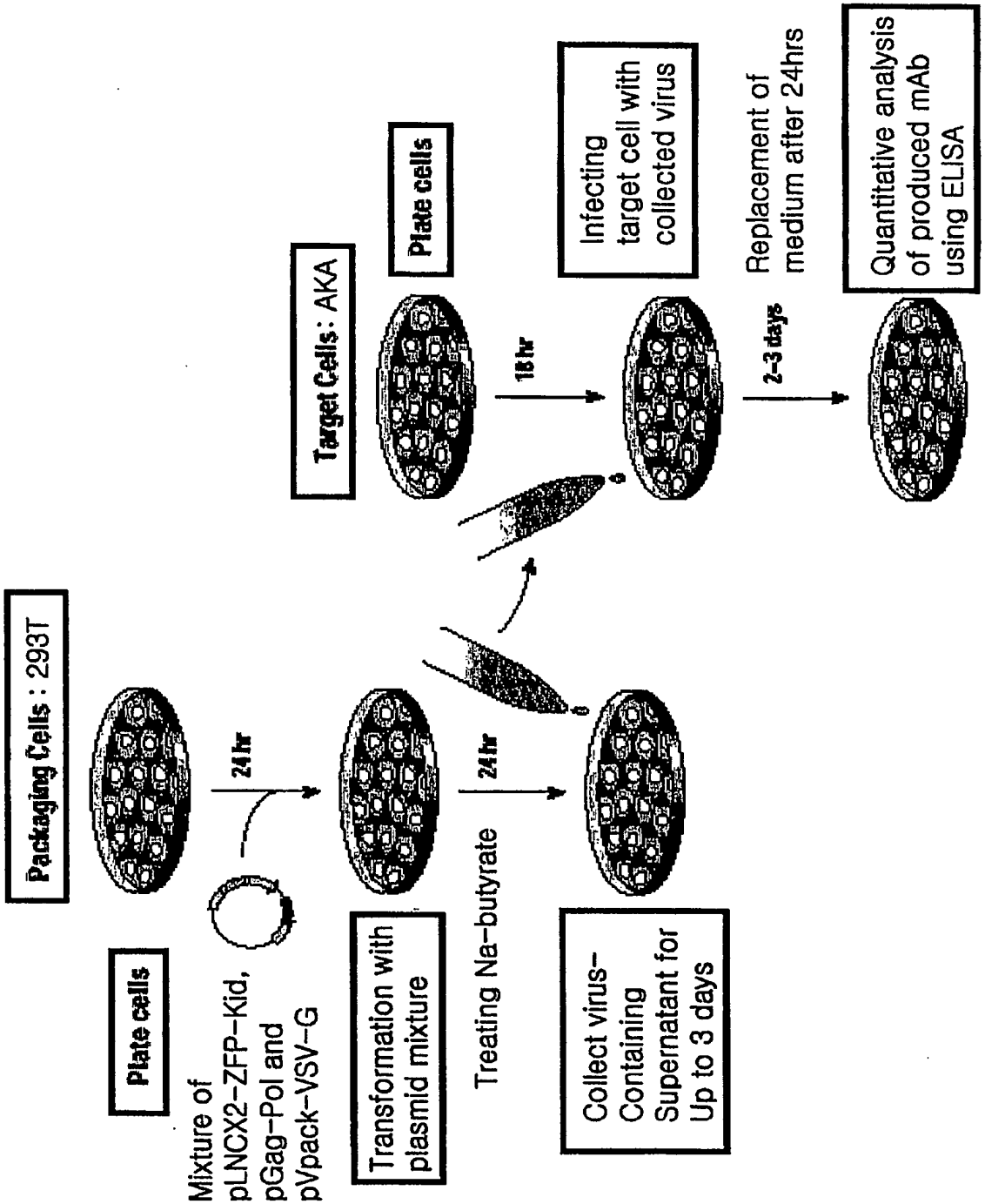
Fig. 2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Transformation with plasmid mixture
24hr
Treating Na-butyrate
Collect virus-Containing Supernatant for Up to 3 days
Target Cells : AKA
Plate cells
18hr
Infecting target cell with collected virus
2-3 days
Replacement of medium after 24hrs
Quantitative analysis of produced mAb using ELISA

Fig. 3

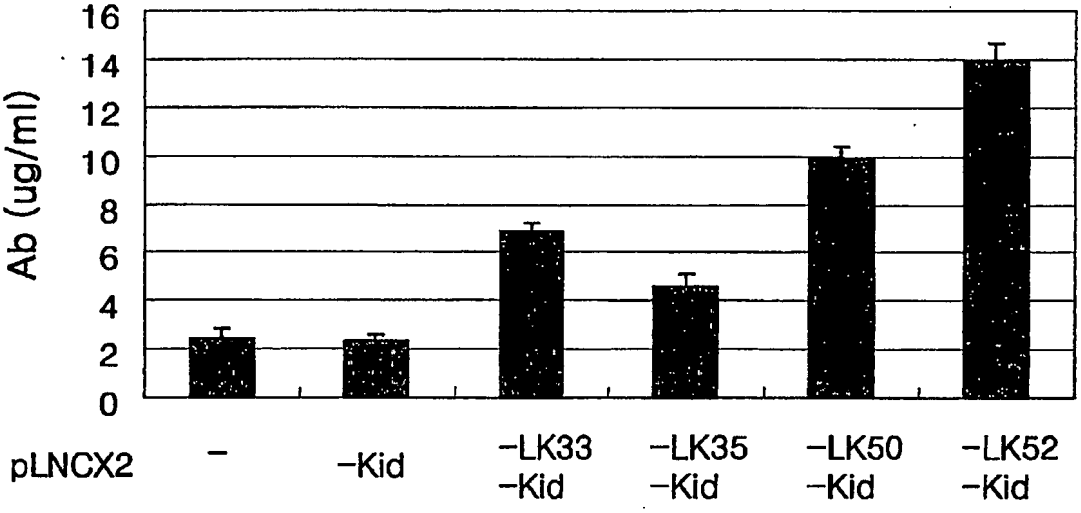
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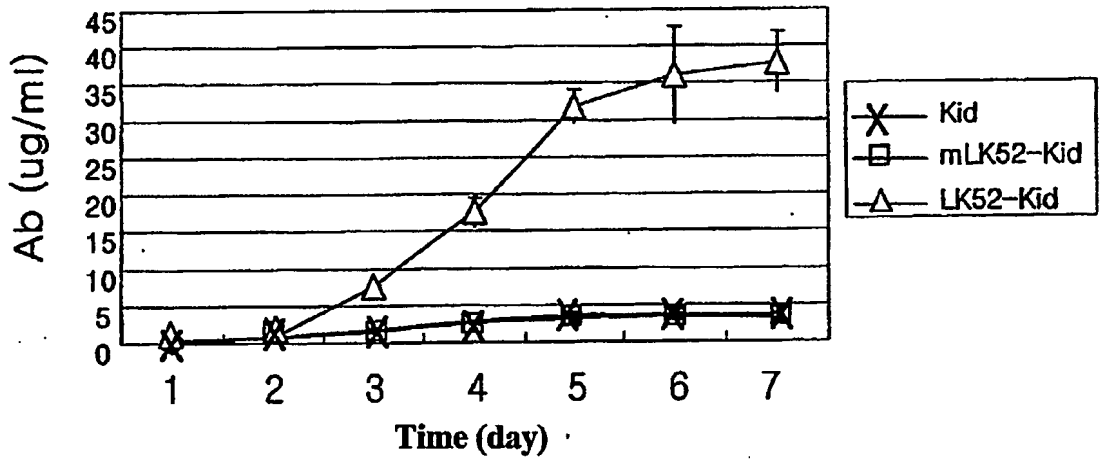
Fig. 4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2
3
4
5
6
7
Time (day)
Kid
mLK52-Kid
LK52-Kid

Fig. 5

P3- -----AAGCT TGCCACC ATG GTG TAC CCC TAC GAC GTG CCC GAC TAC GCC

HindIII Met V Y P Y D V P D Y A (HA tag)

BamHI *XmaI*

GAA TTG CCT CCA AAA AAG AAG AAG AGA AAG GTA GGG ATC CGA ATT CCC GGG GAA AAA CCG

P P K K K R K V (NLS) *EcoRI* P G E K P

AgeI *NotI* *XhoI*

- F1 - ACC GGT GAA AAA GCG GCC GCA TGA GAT CTC GAG ----P3

T G E K A A A (Stop)

Fig. 6

LNCX2----AAGCT TGCCACC **ATG** GTG TAC CCC TAC GAC GTG CCC GAC TAC GCC GAA TTG

*Hind*III **Met**

CCT CCA AAA AAG AAG AGA AAG GTA GGG ATC CGA ATT CCC GCG GCC GCT AAA TTC GTG

*Bam*HI *Eco*RI *Not*I →*Kid-1*

TCA GTG ACA TTT GAA GAT GTG GCT GTG CTC TTT ACT CGG GAC GAG TGG AAG AAG CTG

GAT CTG TCT CAG AGA AGC CTG TAC CGT GAG GTG ATG CTG GAG AAT TAC AGC AAC CTG

GCC TCC ATG GCA GGA TTC CTG TTT ACC AAA CCA AAG GTG ATC TCC CTG TTG CAG CAA

GGA GAG GAT CCC TGG **TAA** CTC GAG CATGCATCTAGA GCGAT----LNCX2

*Bam*HI **(Stop)** *Xho*I (*Apa*I/*Cla*I)

Fig. 7

LNCX2----AAGCT TGCCACC **ATG** GTG TAC CCC TAC GAC GTG CCC GAC TAC GCC GAA TTG CCT

*Hind*III **Met**

CCA AAA AAG AAG AGA AAG GTA GGG ATC CGA ATT CCC GGG GAA AAA CCG **-F1- ACC GGT GAA**

*Bam*HI *Eco*RI *Xma*I

AAA CCG - F2- ACC GGT GAA AAA CCG -F3- ACC GGT GAA AAA CCG -F4- ACC GGT GAA AAA

*Age*I

GCC GCG GCC GCT AAA TTC GTG TCA GTG ACA TTT GAA GAT GTG GCT GTG CTC TTT ACT CGG

*Not*I →*Kid-1*

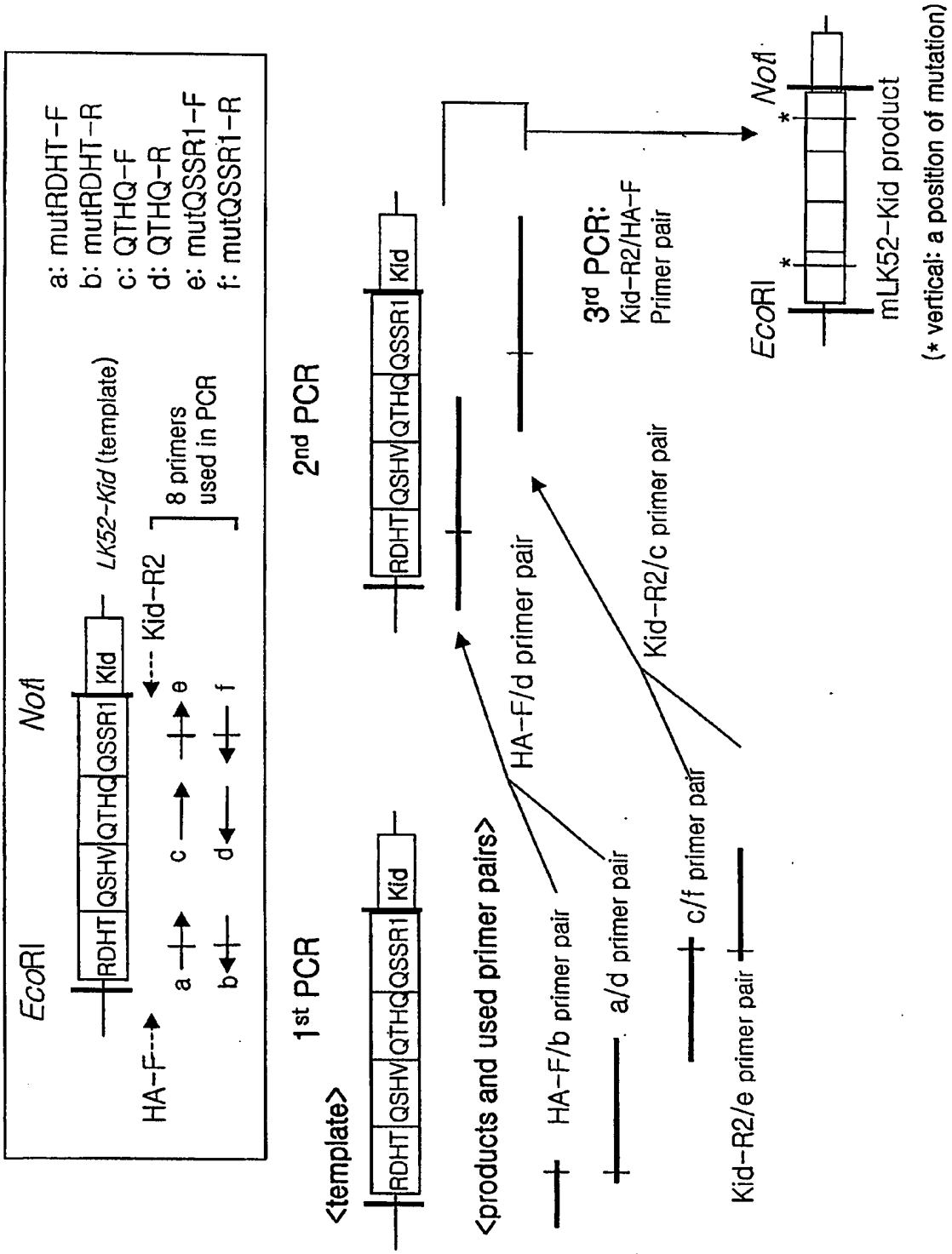
GAC GAG TGG AAG AAG CTG GAT CTG TCT CAG AGA AGC CTG TAC CGT GAG GTG ATG CTG GAG

AAT TAC AGC AAC CTG GCC TCC ATG GCA GGA TTC CTG TTT ACC AAA CCA AAG GTG ATC TCC

CTG TTG CAG CAA GGA GAG GAT CCC TGG **TAA** CTC GAG CATGCATCTAGA GCGAT----LNCX2

*Bam*HI **(Stop)** *Xho*I (*Apa*I/*Cla*I)

Fig. 8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NotI
RDHT
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QTHQ
QSSR1
Kid
LK52-Kid (template)
HA-F
Kid-R2
a
b
c
d
e
f
8 primers used in PCR
a: mutRDHT-F
b: mutRDHT-R
c: QTHQ-F
d: QTHQ-R
e: mutQSSR1-F
f: mutQSSR1-R
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2nd PCR
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a/d primer pair
HA-F/d primer pair
Kid-R2/c primer pair
c/f primer pair
Kid-R2/e primer pair
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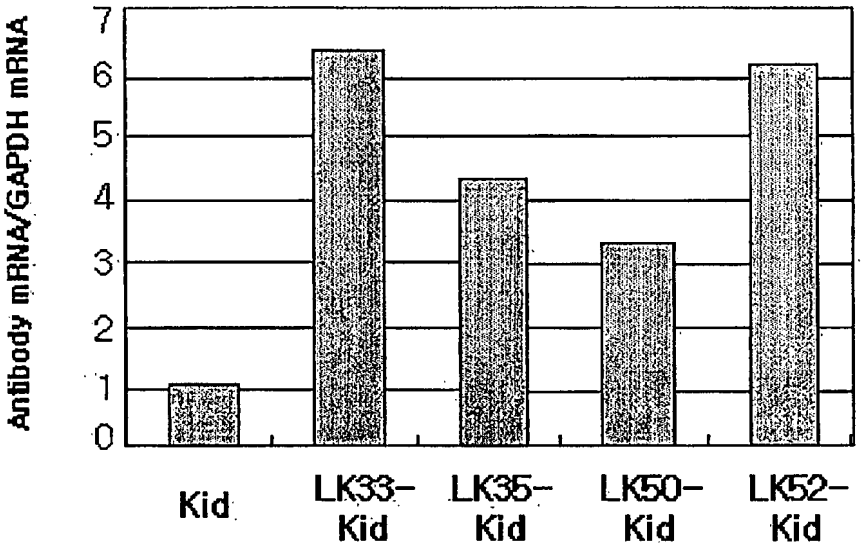
Kid-R2/HA-F
Primer pair
EcoRI
NotI
mLK52-Kid product
(* vertical: a position of mutation)

Fig. 9


Antibody mRNA/GAPDH mRNA
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1
2
3
4
5
6
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Kid
LK33-Kid
LK35-Kid
LK50-Kid
LK52-Kid

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20 25

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35 40 45

Arg Ile His Thr Gly Glu Lys Pro Tyr Lys Cys Glu Glu Cys Gly Lys
50 55 60

Ala Phe Thr Gln Ser Ser Asn Leu Thr Lys His Lys Lys Ile His Thr
65 70 75 80

Gly Glu Lys Pro Tyr Lys Cys Pro Asp Cys Gly Lys Ser Phe Ser Gln
85 90 95

Ser Ser Ser Leu Ile Arg His Gln Arg Thr His Thr Gly Glu Lys
100 105 110

<210> 13
<211> 111
<212> PRT
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<220>
<223> ZFP LK35

<400> 13
Phe Gln Cys Lys Thr Cys Gln Arg Lys Phe Ser Arg Ser Asp His Leu
1 5 10 15

Lys Thr His Thr Arg Thr His Thr Gly Glu Lys Pro Phe Glu Cys Lys
20 25 30

Asp Cys Gly Lys Ala Phe Ile Gln Lys Ser Asn Leu Ile Arg His Gln
35 40 45

Arg Thr His Thr Gly Glu Lys Pro Tyr Glu Cys His Asp Cys Gly Lys
50 55 60

Ser Phe Arg Gln Ser Thr His Leu Thr Arg His Arg Arg Ile His Thr
65 70 75 80

Gly Glu Lys Pro Tyr Met Cys Ser Glu Cys Gly Arg Gly Phe Ser Gln
85 90 95

Lys Ser Asn Leu Thr Ile His Gln Arg Thr His Thr Gly Glu Lys
100 105 110

<210> 14
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> ZFP LK50

<400> 14
Tyr Glu Cys His Asp Cys Gly Lys Ser Phe Arg Gln Ser Thr His Leu
1 5 10 15

Thr Arg His Arg Arg Ile His Thr Gly Glu Lys Pro Tyr Val Cys Asp
20 25 30

Val Glu Gly Cys Thr Trp Lys Phe Ala Arg Ser Asp Lys Leu Asn Arg
35 40 45

His Lys Lys Arg His Thr Gly Glu Lys Pro Phe Gln Cys Lys Thr Cys
50 55 60

Gln Arg Lys Phe Ser Arg Ser Asp His Leu Lys Thr His Thr Arg Thr
65 70 75 80

His Thr Gly Glu Lys Pro Tyr Glu Cys His Asp Cys Gly Lys Ser Phe
85 90 95

Arg Gln Ser Thr His Leu Thr Arg His Arg Arg Ile His Thr Gly Glu
100 105 110
Lys

<210> 15
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> ZFP LK52

<400> 15
Phe Gln Cys Lys Thr Cys Gln Arg Lys Phe Ser Arg Ser Asp His Leu
1 5 10 15

Lys Thr His Thr Arg Thr His Thr Gly Glu Lys Pro Tyr Glu Cys Asp
20 25 30

His Cys Gly Lys Ser Phe Ser Gln Ser Ser His Leu Asn Val His Lys
35 40 45

Arg Thr His Thr Gly Glu Lys Pro Tyr Glu Cys His Asp Cys Gly Lys
50 55 60

Ser Phe Arg Gln Ser Thr His Leu Thr Gln His Arg Arg Ile His Thr
65 70 75 80

Gly Glu Lys Pro Tyr Lys Cys Pro Asp Cys Gly Lys Ser Phe Ser Gln
85 90 95

Ser Ser Ser Leu Ile Arg His Gln Arg Thr His Thr Gly Glu Lys
100 105 110

<210> 16
<211> 9
<212> PRT
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<220>
<223> HA-tag

<400> 16
Tyr Pro Tyr Asp Val Pro Asp Tyr Ala
1 5

<210> 17
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
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<400> 17
Pro Pro Lys Lys Lys Arg Lys Val
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<210> 18
<211> 69
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(69)
<223> ZFD CSNR1

<400> 18
tat aaa tgt aag caa tgt ggg aag gct ttt gga tgt ccc tca aac ctt 48
Tyr Lys Cys Lys Gln Cys Gly Lys Ala Phe Gly Cys Pro Ser Asn Leu
1 5 10 15

cga agg cat gga agg act cac 69
Arg Arg His Gly Arg Thr His
20

<210> 19
<211> 23
<212> PRT
<213> Homo sapiens

<400> 19
Tyr Lys Cys Lys Gln Cys Gly Lys Ala Phe Gly Cys Pro Ser Asn Leu
1 5 10 15

Arg Arg His Gly Arg Thr His
20

<210> 20
<211> 69
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(69)
<223> ZFD DSAR2

<400> 20
tac tcc tgt ggc att tgt ggc aaa tcc ttc tct gac tcc agt gcc aaa 48
Tyr Ser Cys Gly Ile Cys Gly Lys Ser Phe Ser Asp Ser Ser Ala Lys
1 5 10 15

agg aga cac tgc att cta cac 69
Arg Arg His Cys Ile Leu His
20

<210> 21
<211> 23
<212> PRT
<213> Homo sapiens

<400> 21
Tyr Ser Cys Gly Ile Cys Gly Lys Ser Phe Ser Asp Ser Ser Ala Lys
1 5 10 15

Arg Arg His Cys Ile Leu His
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<210> 22

<211> 69
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(69)
<223> ZFD DSCR

<400> 22
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Tyr Thr Cys Ser Asp Cys Gly Lys Ala Phe Arg Asp Lys Ser Cys Leu
1 5 10 15

aac aga cat cgg aga act cat 69
Asn Arg His Arg Arg Thr His
20

<210> 23
<211> 23
<212> PRT
<213> Homo sapiens

<400> 23
Tyr Thr Cys Ser Asp Cys Gly Lys Ala Phe Arg Asp Lys Ser Cys Leu
1 5 10 15

Asn Arg His Arg Arg Thr His
20

<210> 24
<211> 69
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(69)
<223> ZFD DGNV

<400> 24
ttt caa tgt cgt att tgt atg cgt aat ttt tct gat tct ggt aat ttg 48
Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser Asp Ser Gly Asn Leu
1 5 10 15

cgt gtt cat att cgt act cat 69
Arg Val His Ile Arg Thr His
20

<210> 25
<211> 23
<212> PRT
<213> Homo sapiens

<400> 25
Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser Asp Ser Gly Asn Leu
1 5 10 15

Arg Val His Ile Arg Thr His
20

<210> 26
<211> 75
<212> DNA
<213> Homo sapiens

<220>
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<222> (1)..(75)
<223> FPD DSNR

<400> 26
tat gct tgc cct gtc gag tcc tgc gat cgc cgc ttt tct gat tcg tcg 48
Tyr Ala Cys Pro Val Glu Ser Cys Asp Arg Arg Phe Ser Asp Ser Ser
1 5 10 15

aac ctt acc cgc cat atc cgc atc cac 75
Asn Leu Thr Arg His Ile Arg Ile His
20 25

<210> 27
<211> 25
<212> PRT
<213> Homo sapiens

<400> 27
Tyr Ala Cys Pro Val Glu Ser Cys Asp Arg Arg Phe Ser Asp Ser Ser
1 5 10 15

Asn Leu Thr Arg His Ile Arg Ile His

20 25

<210> 28
<211> 72
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(72)
<223> ZFD ISNR

<400> 28
tac agg tgt aag tac tgc gac cgc tcc ttc agc atc tct tcg aac ctc 48
Tyr Arg Cys Lys Tyr Cys Asp Arg Ser Phe Ser Ile Ser Ser Asn Leu
1 5 10 15

cag cgg cac gtc cgg aac atc cac 72
Gln Arg His Val Arg Asn Ile His
20

<210> 29
<211> 24
<212> PRT
<213> Homo sapiens

<400> 29
Tyr Arg Cys Lys Tyr Cys Asp Arg Ser Phe Ser Ile Ser Ser Asn Leu
1 5 10 15

Gln Arg His Val Arg Asn Ile His
20

<210> 30
<211> 69
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(69)
<223> ZFD QFNR

<400> 30

tat aag tgt cat caa tgt ggg aaa gcc ttt att caa tcc ttt aac ctt 48
Tyr Lys Cys His Gln Cys Gly Lys Ala Phe Ile Gln Ser Phe Asn Leu
1 5 10 15

cga aga cat gag aga act cac 69
Arg Arg His Glu Arg Thr His
20

<210> 31
<211> 23
<212> PRT
<213> Homo sapiens

<400> 31
Tyr Lys Cys His Gln Cys Gly Lys Ala Phe Ile Gln Ser Phe Asn Leu
1 5 10 15

Arg Arg His Glu Arg Thr His
20

<210> 32
<211> 69
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(69)
<223> ZFD QSHV

<400> 32
tat gag tgt gat cac tgt gga aaa tcc ttt agc cag agc tct cat ctg 48
Tyr Glu Cys Asp His Cys Gly Lys Ser Phe Ser Gln Ser Ser His Leu
1 5 10 15

aat gtg cac aaa aga act cac 69
Asn Val His Lys Arg Thr His
20

<210> 33
<211> 23
<212> PRT
<213> Homo sapiens

<400> 33

Tyr Glu Cys Asp His Cys Gly Lys Ser Phe Ser Gln Ser Ser His Leu
1 5 10 15

Asn Val His Lys Arg Thr His
20

<210> 34
<211> 69
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(69)
<223> ZFD QSNI

<400> 34
tac atg tgc agt gag tgt ggg cga ggc ttc agc cag aag tca aac ctc 48
Tyr Met Cys Ser Glu Cys Gly Arg Gly Phe Ser Gln Lys Ser Asn Leu
1 5 10 15

acc ata cac cag agg aca cac 69
Thr Ile His Gln Arg Thr His
20

<210> 35
<211> 23
<212> PRT
<213> Homo sapiens

<400> 35
Tyr Met Cys Ser Glu Cys Gly Arg Gly Phe Ser Gln Lys Ser Asn Leu
1 5 10 15

Thr Ile His Gln Arg Thr His
20

<210> 36
<211> 69
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(69)

<223> ZFD QSNK

<400> 36
tac aag tgt gaa gaa tgt ggc aag gct ttt acc caa tcc tca aac ctt 48
Tyr Lys Cys Glu Glu Cys Gly Lys Ala Phe Thr Gln Ser Ser Asn Leu
1 5 10 15

act aaa cat aag aaa att cat 69
Thr Lys His Lys Lys Ile His
20

<210> 37
<211> 23
<212> PRT
<213> Homo sapiens

<400> 37
Tyr Lys Cys Glu Glu Cys Gly Lys Ala Phe Thr Gln Ser Ser Asn Leu
1 5 10 15

Thr Lys His Lys Lys Ile His
20

<210> 38
<211> 69
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(69)
<223> ZFD QSNR1

<400> 38
ttt gag tgt aaa gat tgc ggg aag gct ttc att cag aag tca aac ctc 48
Phe Glu Cys Lys Asp Cys Gly Lys Ala Phe Ile Gln Lys Ser Asn Leu
1 5 10 15

atc aga cac cag aga act cac 69
Ile Arg His Gln Arg Thr His
20

<210> 39
<211> 23

<212> PRT
<213> Homo sapiens

<400> 39
Phe Glu Cys Lys Asp Cys Gly Lys Ala Phe Ile Gln Lys Ser Asn Leu
1 5 10 15

Ile Arg His Gln Arg Thr His
20

<210> 34
<211> 69
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(69)
<223> ZFD QSNV2

<400> 40
tat gtt tgc tca aaa tgt ggg aaa gcc ttc act cag agt tca aat ctg 48
Tyr Val Cys Ser Lys Cys Gly Lys Ala Phe Thr Gln Ser Ser Asn Leu
1 5 10 15

act gta cat caa aaa atc cac 69
Thr Val His Gln Lys Ile His
20

<210> 41
<211> 23
<212> PRT
<213> Homo sapiens

<400> 41
Tyr Val Cys Ser Lys Cys Gly Lys Ala Phe Thr Gln Ser Ser Asn Leu
1 5 10 15

Thr Val His Gln Lys Ile His
20

<210> 42
<211> 69
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(69)
<223> ZFD QSSR1

<400> 42
tat aag tgc cct gat tgt ggg aag agt ttt agt cag agt tcc agc ctc 48
Tyr Lys Cys Pro Asp Cys Gly Lys Ser Phe Ser Gln Ser Ser Ser Leu
1 5 10 15

att cgc cac cag cgg aca cac 69
Ile Arg His Gln Arg Thr His
20

<210> 43
<211> 23
<212> PRT
<213> Homo sapiens

<400> 43
Tyr Lys Cys Pro Asp Cys Gly Lys Ser Phe Ser Gln Ser Ser Ser Leu
1 5 10 15

Ile Arg His Gln Arg Thr His
20

<210> 44
<211> 69
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(69)
<223> ZFD QTHQ

<400> 44
tat gag tgt cac gat tgc gga aag tcc ttt agg cag agc acc cac ctc 48
Tyr Glu Cys His Asp Cys Gly Lys Ser Phe Arg Gln Ser Thr His Leu
1 5 10 15

act cag cac cgg aga atc cac 69
Thr Gln His Arg Arg Ile His
20

<210> 45
<211> 23
<212> PRT
<213> Homo sapiens

<400> 45
Tyr Glu Cys His Asp Cys Gly Lys Ser Phe Arg Gln Ser Thr His Leu
1 5 10 15

Thr Gln His Arg Arg Ile His
20

<210> 46
<211> 69
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(69)
<223> ZFD QTHR1

<400> 46
tat gag tgt cac gat tgc gga aag tcc ttt agg cag agc acc cac ctc 48
Tyr Glu Cys His Asp Cys Gly Lys Ser Phe Arg Gln Ser Thr His Leu
1 5 10 15

act cgg cac cgg aga atc cac 69
Thr Arg His Arg Arg Ile His
20

<210> 47
<211> 23
<212> PRT
<213> Homo sapiens

<400> 47
Tyr Glu Cys His Asp Cys Gly Lys Ser Phe Arg Gln Ser Thr His Leu
1 5 10 15

Thr Arg His Arg Arg Ile His
20

<210> 48
<211> 75
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(75)
<223> ZFD RDER1

<400> 48
tat gta tgc gat gta gag gga tgt acg tgg aaa ttt gcc cgc tca gat 48
Tyr Val Cys Asp Val Glu Gly Cys Thr Trp Lys Phe Ala Arg Ser Asp
1 5 10 15

gag ctc aac aga cac aag aaa agg cac 75
Glu Leu Asn Arg His Lys Lys Arg His
20 25

<210> 49
<211> 25
<212> PRT
<213> Homo sapiens

<400> 49
Tyr Val Cys Asp Val Glu Gly Cys Thr Trp Lys Phe Ala Arg Ser Asp
1 5 10 15

Glu Leu Asn Arg His Lys Lys Arg His
20 25

<210> 50
<211> 69
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(69)
<223> ZFD RDHT

<400> 50
ttc cag tgt aaa act tgt cag cga aag ttc tcc cgg tcc gac cac ctg 48
Phe Gln Cys Lys Thr Cys Gln Arg Lys Phe Ser Arg Ser Asp His Leu
1 5 10 15

aag acc cac acc agg act cat 69
Lys Thr His Thr Arg Thr His
20

<210> 51
<211> 23
<212> PRT
<213> Homo sapiens

<400> 51
Phe Gln Cys Lys Thr Cys Gln Arg Lys Phe Ser Arg Ser Asp His Leu
1 5 10 15

Lys Thr His Thr Arg Thr His
20

<210> 52
<211> 75
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(75)
<223> ZFD RDKR

<400> 52
tat gta tgc gat gta gag gga tgt acg tgg aaa ttt gcc cgc tca gat 48
Tyr Val Cys Asp Val Glu Gly Cys Thr Trp Lys Phe Ala Arg Ser Asp
1 5 10 15

aag ctc aac aga cac aag aaa agg cac 75
Lys Leu Asn Arg His Lys Lys Arg His
20 25

<210> 53
<211> 25
<212> PRT
<213> Homo sapiens

<400> 53
Tyr Val Cys Asp Val Glu Gly Cys Thr Trp Lys Phe Ala Arg Ser Asp
1 5 10 15

Lys Leu Asn Arg His Lys Lys Arg His
20 25

<210> 54
<211> 69
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(69)
<223> ZFD RDNQ

<400> 54
ttt gcc tgc cct gag tgt cct aag cgc ttc atg aga tcc gac aac ctg 48
Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met Arg Ser Asp Asn Leu
1 5 10 15

acc cag cat atc aag acc cac 69
Thr Gln His Ile Lys Thr His
20

<210> 55
<211> 23
<212> PRT
<213> Homo sapiens

<400> 55
Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met Arg Ser Asp Asn Leu
1 5 10 15

Thr Gln His Ile Lys Thr His
20

<210> 56
<211> 69
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(69)
<223> ZFD RSHR

<400> 56
tat aag tgc atg gag tgt ggg aag gct ttt aac cgc agg tca cac ctc 48
Tyr Lys Cys Met Glu Cys Gly Lys Ala Phe Asn Arg Arg Ser His Leu
1 5 10 15

aca cgg cac cag cgg att cac 69
Thr Arg His Gln Arg Ile His
20

<210> 57
<211> 23
<212> PRT
<213> Homo sapiens

<400> 57
Tyr Lys Cys Met Glu Cys Gly Lys Ala Phe Asn Arg Arg Ser His Leu
1 5 10 15

Thr Arg His Gln Arg Ile His
20

<210> 58
<211> 69
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(69)
<223> ZFD RSNR

<400> 58
tat att tgc aga aag tgt gga cgg ggc ttt agt cgg aag tcc aac ctt 48
Tyr Ile Cys Arg Lys Cys Gly Arg Gly Phe Ser Arg Lys Ser Asn Leu
1 5 10 15

atc aga cat cag agg aca cac 69
Ile Arg His Gln Arg Thr His
20

<210> 59
<211> 23
<212> PRT
<213> Homo sapiens

<400> 59
Tyr Ile Cys Arg Lys Cys Gly Arg Gly Phe Ser Arg Lys Ser Asn Leu
1 5 10 15

Ile Arg His Gln Arg Thr His
20

<210> 60
<211> 69
<212> DNA
<213> Homo sapiens

<220>
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<222> (1)..(69)
<223> ZFD VSNV

<400> 60
tat gaa tgc gat cac tgt ggg aaa gcc ttc agc gtc agc tcc aac ctg 48
Tyr Glu Cys Asp His Cys Gly Lys Ala Phe Ser Val Ser Ser Asn Leu
1 5 10 15

aac gtg cac aga aga atc cac 69
Asn Val His Arg Arg Ile His
20

<210> 61
<211> 23
<212> PRT
<213> Homo sapiens

<400> 61
Tyr Glu Cys Asp His Cys Gly Lys Ala Phe Ser Val Ser Ser Asn Leu
1 5 10 15

Asn Val His Arg Arg Ile His
20

<210> 62
<211> 69
<212> DNA
<213> Homo sapiens

<220>
<221> CDS

<222> (1)..(69)
<223> ZFD VSSR

<400> 62
tat aca tgt aaa cag tgt ggg aaa gcc ttc agt gtt tcc agt tcc ctt 48
Tyr Thr Cys Lys Gln Cys Gly Lys Ala Phe Ser Val Ser Ser Ser Leu
1 5 10 15

cga aga cat gaa acc act cac 69
Arg Arg His Glu Thr Thr His
20

<210> 63
<211> 23
<212> PRT
<213> Homo sapiens

<400> 63
Tyr Thr Cys Lys Gln Cys Gly Lys Ala Phe Ser Val Ser Ser Ser Leu
1 5 10 15

Arg Arg His Glu Thr Thr His
20

<210> 64
<211> 69
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(69)
<223> ZFD VSTR

<400> 64
tat gag tgt aat tac tgt gga aaa acc ttt agt gtg agc tca acc ctt 48
Tyr Glu Cys Asn Tyr Cys Gly Lys Thr Phe Ser Val Ser Ser Thr Leu
1 5 10 15

att aga cat cag aga atc cac 69
Ile Arg His Gln Arg Ile His
20

<210> 65

<211> 23
<212> PRT
<213> Homo sapiens

<400> 65
Tyr Glu Cys Asn Tyr Cys Gly Lys Thr Phe Ser Val Ser Ser Thr Leu
1 5 10 15

Ile Arg His Gln Arg Ile His
20

<210> 66
<211> 69
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(69)
<223> ZFD WSNR

<400> 66
tac aga tgt gag gaa tgt ggc aaa gcc ttt agg tgg ccc tca aac ctt 48
Tyr Arg Cys Glu Glu Cys Gly Lys Ala Phe Arg Trp Pro Ser Asn Leu
1 5 10 15

act aga cat aag aga ata cac 69
Thr Arg His Lys Arg Ile His
20

<210> 67
<211> 23
<212> PRT
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<400> 67
Tyr Arg Cys Glu Glu Cys Gly Lys Ala Phe Arg Trp Pro Ser Asn Leu
1 5 10 15

Thr Arg His Lys Arg Ile His
20

<210> 68
<211> 189
<212> DNA

<213> Rattus sp.

<400> 68
gtgtcagtga catttgaaga tgtggctgtg ctctttactc gggacgagtg gaagaagctg 60

gatctgtctc agagaagcct gtaccgtgag gtgatgctgg agaattacag caacctggcc 120

tccatggcag gattcctgtt taccaaacca aaggtgatct ccctgttgca gcaaggagag 180

gatccctgg 189

<210> 69
<211> 63
<212> PRT
<213> Rattus sp.

<220>
<221> DOMAIN
<222> (1)..(63)
<223> Kid transcription repression domain

<400> 69
Val Ser Val Thr Phe Glu Asp Val Ala Val Leu Phe Thr Arg Asp Glu
1 5 10 15

Trp Lys Lys Leu Asp Leu Ser Gln Arg Ser Leu Tyr Arg Glu Val Met
20 25 30

Leu Glu Asn Tyr Ser Asn Leu Ala Ser Met Ala Gly Phe Leu Phe Thr
35 40 45

Lys Pro Lys Val Ile Ser Leu Leu Gln Gln Gly Glu Asp Pro Trp
50 55 60

<210> 70
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligomer LNCX1-F

<220>

<221> mutation
<222> (15)

<400> 70
taggcgccgg aatttcgatc tgatcaaga 29

<210> 71
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
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<220>
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<222> (15)

<400> 71
tcttgatcag atcgaaattc cggcgccta 29

<210> 72
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligomer Kid-F

<400> 72
gggcggccgc taaattcgtg tcagtgaca 29

<210> 73
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligomer Kid-R1

<400> 73
ccgctcgagt taccagggat cctctcc 27

<210> 74
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Target site of ZFP LK33

<220>
<221> variation
<222> (10)
<223> A or G or C or T

<400> 74
gyadaavgan gg 12

<210> 75
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Target site of ZFP LK35

<220>
<221> variation
<222> (10)
<223> A or G or C or T

<400> 75
maavgagaan gg 12

<210> 76
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Target site of ZFP LK50

<220>

<221> variation
<222> (4)
<223> A or G or C or T

<400> 76
vganggrggv ga 12

<210> 77
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Target site of ZFP LK52

<220>
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<222> (10)
<223> A or G or C or T

<400> 77
gyahgahgan gg 12

<210> 78
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligomer NLS-F

<400> 78
cctccaaaaa agaagagaaa ggta 24

<210> 79
<211> 27
<212> DNA
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<220>
<223> Oligomer mutRDHT-R

<220>
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<400> 79
caggtggtcg gaggcggaga actttcg 27

<210> 80
<211> 27
<212> DNA
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<220>
<223> mutRDHT-F

<220>
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<222> (13)..(15)

<400> 80
cgaaagttct ccgcctccga ccacctg 27

<210> 81
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligomer QTHQ-R

<400> 81
cctaaaggac tttccgcaat cgtgacactc 30

<210> 82
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligomer QTHQ-F

<400> 82

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2005/001541

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C07K 14/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 : C07K 14/00, C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubMed, NCBI, eKIPASS, Delphion

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y A	Wang, J.H., et al. "Aiolos regulates B cell activation and maturation to effector state" Immunity, Oct. 1998, Vol. 9(4): pages 543-553, See entire document.	1, 5-11, 14-17, 20, 21 2-4, 12, 13, 18, 19
Y A	Bae, K.H., et al. "Human zinc fingers as building blocks in the construction of artificial transcription factors" Nature Biotech., Mar. 2003, Vol. 21: pags 275-280, See entire document.	1, 5-11, 14-17, 20, 21 2-4, 12, 13, 18, 19
A	Radulescu, R.T. "Antibody constant region: potential to bind metal and nucleic acid" Med Hypotheses, Feb. 1995, Vol. 44(2): pages 139-145, See entire document.	1-21
A	Witzgall, R., et al. "Kid-1 expression is high in differencetiated renal proximal tubule cells and suppressed in cyst epithelia" Am J Physiol., Dec. 1998, Vol. 275(6 Pt 2): pages F928-937, See entire document.	1-21

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 AUGUST 2005 (30.08.2005)	**30 AUGUST 2005 (30.08.2005)**

Name and mailing address of the ISA/KR	Authorized officer
Korean Intellectual Property Office 920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea	PARK, JEONG UNG
Facsimile No. 82-42-472-7140	Telephone No. 82-42-481-8159

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2005/001541

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of :

   a. type of material
      - [x] a sequence listing
      - [x] table(s) related to the sequence listing

   b. format of material
      - [x] on paper
      - [x] in electronic form

   c. time of filing/furnishing
      - [x] contained in the international application as filed
      - [x] filed together with the international application in electronic form
      - [ ] furnished subsequently to this Authority for the purposes of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments: